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Advances in Organic Chemistry

Methods and Results

VOLUME I

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Advances in Organic Chemistry

Methods and Results

VOLUME I

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Introduction to the Series

Although it must be one of the most thoroughly documented of the sciences, the subject of organic chemistry exercises a perennial fascination for the scientific publisher. Any addition to this highly populated sphere would seem to require a word of explanation if not justification.

In blatant disregard of all the predictions of its impending exhaustion, organic chemistry continues to expand at a headlong rate. Frequently this rapidity of evolution creates a time lag between the elaboration of useful new methods and ideas and their appreciation and general use by other organic chemists. There appeared to be scope for a series of volumes containing articles providing a critical appraisal and evaluation of new aspects ripe for further development, and of novel extensions to well-established methods.

It is one thing to enunciate such a venture in high-sounding terms; it is quite another to persuade contributors of standing to accept the hard realities of authorship. Ideally the author of such an article should be either the originator of the method described or a practitioner expert in its use. To some extent this criterion is self-defeating, as there is still lingering agreement with the dictum "Those who can, do. Those who can't, write reviews." However, by a judicious mixture of persuasion and bullying, this desirable arrangement has been achieved in the present series, and we wish to thank the authors most sincerely for their valued collaboration in this project. They have suffered with remarkably good temper the demands on their valuable time, our rude remarks on their manuscripts, and, perhaps most trying of all, those seemingly inevitable delays attached to scientific book production which necessitate considerable new insertions.

Apart from purely mechanical considerations of layout we have made no attempt to dictate the style and presentation of these articles, or to impose that uniform, characterless aridity so beloved of journal editors. Some of the contributions are considerably detailed, others take a bird's-eye view and pick out the highlights; all are personal,

95729

speculative, authoritative, and at times even polemical. From the nature of the project most of the topics, though not all, are methodological in character, and a practical section has been included where appropriate to enhance the value of this aspect.

A series such as this cannot satisfy everyone and we look forward to receiving opinions, criticisms, and suggestions for incorporation into future volumes.

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THE KOLBE ELECTROLYTIC SYNTHESIS

By B. C. L. WEEDON, *Imperial College of Science and Technology, London, England*

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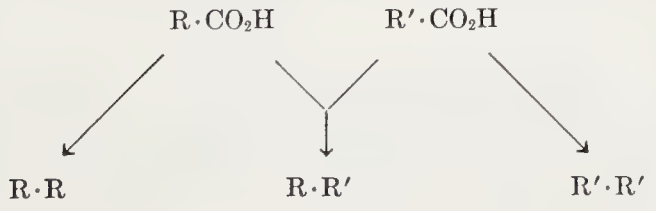
I. Introduction

Of the various reactions of organic compounds at electrodes none has proved more useful for synthesis than that first reported by Kolbe (82) in 1849. This consisted originally in the formation of a symmetrical hydrocarbon and carbon dioxide on electrolysis of a salt of a carboxylic acid:

$$2R\cdot CO_2^- \xrightarrow{-2\epsilon} R\cdot R + 2CO_2$$

In 1855 Wurtz (151) applied the reaction to mixtures of two fatty acids and obtained products by both symmetrical and unsymmetrical (or crossed) coupling of the two components:



This extension of the Kolbe reaction opened the way to the anodic synthesis of a wide variety of unsymmetrical compounds, and was to prove most valuable. Although a mixture of three products is always formed, these can often be separated quite readily, and the yield of the unsymmetrical product frequently compares very favorably with the yields in the alternative, and usually lengthier, routes to the same goal. A wide difference in size of the two coupling units can be tolerated and, in general, the proportions of the three products are those to be expected from the proportions and anodic behavior of the starting materials. A large excess of a readily available acid can therefore be used to promote the conversion of the other component into the required unsymmetrical product (62).

Another important development was the discovery by Brown and Walker (23) in 1891 that half esters of aliphatic dicarboxylic acids, in marked contrast to the dicarboxylic acids themselves (140,144), undergo the Kolbe reaction normally to give diesters:

$$RO_2C\cdot(CH_2)_n\cdot CO_2H \longrightarrow RO_2C\cdot(CH_2)_{2n}\cdot CO_2R$$

In the early investigations on coupling reactions of the Kolbe type aqueous solutions of the reagents were employed, but methanolic solutions have been widely used in recent years. Methanol has the obvious advantage of being a superior solvent for most organic acids. Moreover, the experimental conditions for optimum yields (high carboxylate concentration and anode current density, low temperature and pH of the electrolyte) are less critical in methanolic than in aqueous media (58,62,118,146). A minor practical disadvantage in using methanol as solvent is that the resistance of the cell is greatly increased, and more heat must therefore be dissipated during the electrolysis. Another drawback is that small amounts of the starting materials, $R\cdot CO_2H$, are converted into their methyl esters (17,25,95). However, other side reactions leading to alcohols, ROH (40,44,46,50,52,57,69,85), esters, $R\cdot COOR$ (82,112), and mixtures of paraffins, RH, and the corresponding olefins (62,84,90,125) are much less in evidence in methanolic than in aqueous media (58,62,118).

A convenient general procedure for carrying out reactions of the Kolbe type is to dissolve the starting acid(s) in methanol containing sufficient sodium methoxide to neutralize a small portion (about 2%) of the acid(s), and then to electrolyze the solution between two plat-

inum foil electrodes until it becomes slightly alkaline. As the electrolysis proceeds, the carboxylate ions are converted at the anode into product; the sodium liberated at the cathode reacts with the solvent, and the resulting alkali neutralizes some of the excess acid(s). The process continues in this way until all the starting material has been consumed.

An alternative procedure, recently introduced (32), is to use sufficient sodium methoxide to neutralize the major portion, or all, of the starting material, and to electrolyze the resulting methanolic solution between a platinum anode and a mercury cathode. It is claimed that as long as a potential difference is applied across the electrodes, the electrolyte does not turn alkaline, the excess sodium liberated at the mercury cathode being held there as an amalgam. Owing to the initial high carboxylate ion concentration, large currents can be used with a consequent saving in reaction time. The current drops as the carboxylate ion concentration is reduced by electrolysis, and the termination of the reaction is therefore readily detected.

For preparative electrolyses of the types just described no elaborate apparatus is required. On the laboratory scale the reactions may be carried out quite satisfactorily in a simple cell constructed from a beaker (32,117) or large boiling tube (62), without any special precautions being taken to control the electrode potentials. A semi-technical plant has been developed for continuous operation (13).

By contrast with the numerous studies on aqueous and methanolic media, the electrolysis of salts in an excess of the corresponding anhydrous fatty acid (59,70), and of molten salts (11,99,111), has received very little attention.

The mechanism of the Kolbe reaction has been the subject of much discussion (for a review see 146). It seems most probable that a sequence of three processes is involved at the anode: (*a*) discharge of the carboxylate ions, $R \cdot CO_2^-$, to give carboxylate radicals, (*b*) rapid decarboxylation of the latter with formation of alkyl radicals and carbon dioxide, and (*c*) dimerization of the alkyl radicals to yield the Kolbe product:

$$R \cdot CO_2^- \xrightarrow{-\epsilon} R \cdot CO_2 \cdot \longrightarrow R \cdot + CO_2$$

$$2R \cdot \longrightarrow R \cdot R$$

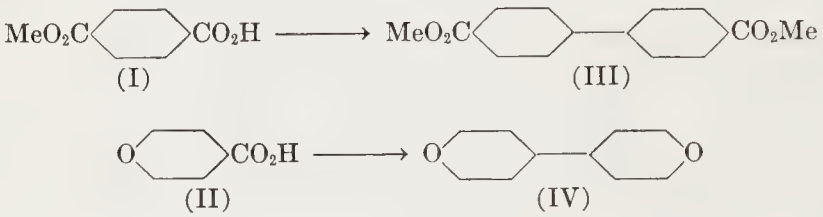
Certainly the side reactions mentioned earlier, and various substitutions (53,55,60,93) and polymerizations (61) that have been observed

during the electrolysis of carboxylic acids, can best be explained on the basis of free radical intermediates. Recent kinetic studies on the electrolysis of acetates and propionates also support the above mechanism (148).

II. Scope and Limitations

Not all acids and half esters undergo the Kolbe synthesis, but the structural features which to some extent limit the scope of the reaction are now fairly well recognized (22,39,134,146).

Electrolyses of aliphatic acids or half esters with unbranched chains of from two to at least eighteen carbon atoms give the expected Kolbe products in yields of about 50–90% (see Tables I and II). However, an alkyl substituent in the α-position to the carboxyl group which is to be eliminated results in a marked suppression of the Kolbe reaction, and then the yield seldom exceeds 10% (cf. 146). Two apparently favorable exceptions are methyl hydrogen cyclohexanedicarboxylate (I) (42) and tetrahydropyrane-4-carboxylic acid (II) (14), which are reported to give the Kolbe products III and IV in 32 and 20% yields, respectively.



Alkyl substituents further removed from the carboxyl group than the α-position seem, in general, to exert little, if any, adverse influence on the Kolbe reaction (38,56,108,109). Of recent years numerous examples have been reported of anodic couplings in high yield with β-mono- and β,β-dialkyl acids and half esters, and with γ-alkyl acids and half esters (see, for example, 3,4,12,27,65,86,88–91,94, 100–102,125–131).

The influence of other substituents in the α-position is often more pronounced than that of an alkyl group. Although coupling occurs with alkyl hydrogen malonates (23), aryloxyacetic acids (43,53), and phenylacetic acid (53,93) and, to a lesser extent, with diphenylacetic acid (114,139), trifluoroacetic acid (136), and a few α-acylamino acids (92), no coupling has been reported for α-halo (34,79,106,137),

TABLE I
Kolbe Reaction with Fatty Acids—$CH_3 \cdot (CH_2)_n \cdot CO_2H$

n	Solvent	Yield, %	Ref.
0	H_2O	90[a]	47
0	MeOH	93[a]	47
1	H_2O	40	39
2	H_2O	60	31
4	H_2O	89	108
10	H_2O–EtOH	90	109
12	MeOH	60	87,147
14	H_2O–EtOH	89	109
14	H_2O–EtOH	60	119
16	H_2O–EtOH	88	109
16	H_2O–EtOH	60	119
18	H_2O–EtOH	73	109

[a] Current yield.

α-hydroxy (142), α-alkoxy (36,142), and α-cyano acids (51). It must, however, be emphasized that the experimental conditions employed in much of the early work on the electrolysis of α-substituted acids cannot now be regarded as having been the most suitable for the Kolbe reaction. A reinvestigation of the electrolysis of simple α-alkoxy and cyano acids, in particular, seems desirable.

TABLE II
Kolbe Reaction with Half Esters—$RO_2C \cdot (CH_2)_n \cdot CO_2H$

R	n	Solvent	Yield, %	Ref.
Et	1	H_2O	60	23
Et	2	H_2O	50	103
Me	2	MeOH	76	18,32
Me	3	MeOH	67	16
Me	4	MeOH	90–95	13
Et	7	MeOH	45	10
Et	8	H_2O	40–55	19,135
Me	8	MeOH	66	62
Et	9	MeOH	~57	117
Me	10	MeOH	43	123,152
Et	10	MeOH		117
Me	11	MeOH	60	115
Me	12	MeOH	~45	152
Me	14	MeOH	68	117(cf.152)
Et	16	H_2O–EtOH	60	37(cf.152)

The electrolysis of a series of ω-halocarboxylic acids in methanol has been studied by Pattison *et al.* (106,107) whose main findings are summarized in Table III. No coupling was observed with ω-halo-

TABLE III
Electrolysis[a] of ω-Halo Acids—$X(CH_2)_n \cdot CO_2H$

	Per cent yield of Kolbe product—$X(CH_2)_{2n}X$			
n	X = F	X = Cl	X = Br	X = I
1	0	0	0	0
2	0	39	0	0
3	0	0	0	0
4	45	51.5	0	0
5	45	55	0	
6	57.5			
7	64		0	
8	65			0
9	69	82	0	0
10	61		54[b]	
15			31	

[a] In MeOH.
[b] Korsching (83) reported 20% from an electrolysis in aqueous alcohol.

acetic, -propionic, or -butyric acids, apart from β-chloropropionic acid, or with any of the ω-iodo acids examined. The difference reported in the behavior of ω-bromodecanoic acid, which underwent no coupling, and ω-bromoundecanoic acid, which gave dibromoeicosane in 54% yield, is remarkable; the much lower yield (20%) of dibromoeicosane obtained by Korsching (83) from an electrolysis in aqueous ethanol seems more in keeping with the other results. It is also interesting to note that a crossed coupling with 6-bromo-3,5-dimethylhexanoic acid was recently reported (5), although Pattison *et al.* observed no (symmetrical) coupling with 6-bromohexanoic acid.

Systematic studies have not yet been made of the influence of other functional substituents in the β- and higher positions, but such information as is available is summarized in Table IV. Many instances have been reported of successful coupling with halo, hydroxy, acetoxy, alkoxy, keto, cyano, amido, and acylamino acids, other than those of the α-series.

From the preceding account it is evident that one of the main limitations of the Kolbe reaction is that it is unsatisfactory with many

TABLE IV
Kolbe Reaction with *O*- and *N*-Substituted Fatty Acids

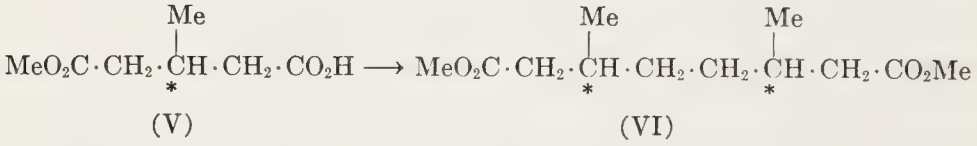
Acid	Solvent	Yield, %	Ref.
$CH_3 \cdot (CH_2)_7 \cdot CHOH \cdot CHOH \cdot (CH_2)_7 \cdot CO_2H$	MeOH	20–50	15
$CH_3 \cdot (CH_2)_5 \cdot CHOH \cdot CHOH \cdot (CH_2)_7 \cdot CO_2H$	MeOH	[a]	17
$HO \cdot (CH_2)_{10} \cdot CO_2H$	H_2O	[a]	133a
$CH_3 \cdot (CH_2)_5 \cdot CHOH \cdot CH_2 \cdot CH{:}CH \cdot (CH_2)_7 \cdot CO_2H$			81
$CH_3 \cdot CHOAc \cdot CH_2 \cdot CO_2H$	MeOH–EtOH	[a]	122
$MeO_2C \cdot CH_2 \cdot CHOAc \cdot CH_2 \cdot CO_2H$	MeOH	[a]	120
$EtO_2C \cdot CHOAc \cdot CH_2 \cdot CO_2H$	MeOH	[a]	71
$AcOCMe_2 \cdot CH_2 \cdot CHMe \cdot CH_2 \cdot CHMe \cdot CH_2 \cdot CO_2H$		[a]	5
$AcO \cdot (CH_2)_{10} \cdot CO_2H$	H_2O	[a]	133a
$MeOCH_2 \cdot CH_2 \cdot CO_2H$	MeOH	[a]	20
$AmOCH_2 \cdot CH_2 \cdot CO_2H$	H_2O		64
$EtOCH_2 \cdot CH_2 \cdot CH_2 \cdot CO_2H$	H_2O	30	104
$EtOCH_2 \cdot CHMe \cdot (CH_2)_3 \cdot CHMe \cdot CH_2 \cdot CH_2 \cdot CO_2H$	MeOH	~54	77
$MeO(CH_2)_9 \cdot CO_2H$	MeOH	[a]	75
$CH_3 \cdot CHOAm \cdot CH_2 \cdot CO_2H$	MeOH	[a]	74
$(EtO)_2CH \cdot CH_2 \cdot CO_2H$	H_2O	60	150
$(EtO)_2CH \cdot CH_2 \cdot CH_2 \cdot CO_2H$	H_2O	35	150
$(EtO)_2CH \cdot (CH_2)_7 \cdot CO_2H$	MeOH		133
$(EtO)_2CH \cdot (CH_2)_8 \cdot CO_2H$	MeOH	52	133
$CH_3 \cdot CO \cdot CH_2 \cdot CH_2 \cdot CO_2H$	H_2O	50	68
$CH_3 \cdot CO \cdot CH_2 \cdot CH_2 \cdot CO_2H$	MeOH	26	24
$CH_3 \cdot CO \cdot (CH_2)_4 \cdot CO_2H$		[a]	132
$CH_3 \cdot CH_2 \cdot CO \cdot (CH_2)_4 \cdot CO_2H$	H_2O	75	45
$CH_3 \cdot CO \cdot CH_2 \cdot CH_2 \cdot CO \cdot CH_2 \cdot CH_2 \cdot CO_2H$	MeOH	[a]	73,74
$CH_3 \cdot CO \cdot CH_2 \cdot CHMe \cdot CH_2 \cdot CHMe \cdot CH_2 \cdot CH_2 \cdot CO_2H$		[a]	5
$CH_3 \cdot CO \cdot (CH_2)_8 \cdot CO_2H$		[a]	132
$CN \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CO_2H$	MeOH	~41	105
$NH_2CO \cdot (CH_2)_4 \cdot CO_2H$	MeOH	14	105
$AcNH \cdot (CH_2)_5 \cdot CO_2H$	MeOH	25–40	105
$PhCONH \cdot (CH_2)_5 \cdot CO_2H$	MeOH		105
$EtSO_2NH \cdot (CH_2)_5 \cdot CO_2H$	MeOH		105

[a] Unsymmetrical coupling reaction.

α-substituted acids. However, there is some indication that, as might be expected, an adverse structural feature may be less deleterious in a crossed coupling reaction, with a component that undergoes the Kolbe reaction readily, than in symmetrical coupling. Thus suc-

cessful crossed coupling has been reported between ethyl hydrogen methylmalonate and 3-methyleicosanoic acid (131), between 2-methyldecanoic acid and ethyl hydrogen sebacate (4,101), and between α-cyano-α-alkyl fatty acids and fatty acids (2). A more thorough investigation of this aspect of the Kolbe reaction might be profitable.

Even when coupling is achieved with an α-substituted acid, any optical activity associated with the α-carbon atom is apparently lost. Thus electrolysis of (−)-α-methylbutyric acid yielded (±)-3,4-dimethylhexane (145). This is not surprising if, as suggested earlier, coupling involves free radicals, and hence a temporary planar distribution at the α-carbon atom. However, an asymmetric center further from the carboxy group is preserved during a Kolbe reaction. This fact was first demonstrated in 1950 by electrolyses of (+)- and (−)-methyl hydrogen β-methylglutarate (V) when the pure



(V) (VI)

(+)- and (−)-forms of methyl β,β'-dimethylsuberate (VI) were obtained (89,126). Subsequently many examples have been published of the retention of optical activity during the symmetrical and unsymmetrical coupling of the enantiomorphs of β-alkylated acids and half esters (see Section III; also 125–131). The preservation of optical activity involving an oxygen substituent has also been demonstrated by electrolyses with the enantiomorphs of β-acetoxybutyric acid (122), methyl hydrogen β-acetoxyglutarate (120), and ethyl hydrogen β-acetoxysuccinate (71).

In the last few years wide use has been made of anodic coupling with optically active intermediates to effect stereospecific syntheses of a variety of branched-chain acids, dibasic acids, hydroxy acids and alcohols (see Section III). An attractive feature of these routes is that they establish a direct stereochemical relationship between the product and the starting material. If, as with all the intermediates cited above, the configuration of the starting material has been correlated with that of D-glyceraldehyde, absolute configurations can be assigned to the product. The suggestion (21) that reliable conclusions concerning the configuration of the product cannot be drawn

from electrolyses with the enantiomorphs of unsymmetrical half esters was based on a misuse of configurational symbols, and is therefore without foundation. Recently it has been shown that anodic coupling of the (+)- and (−)-forms of methyl hydrogen β-methylsuccinate gives products with the expected configuration (125).

An important limitation of the Kolbe reaction is that neither α,β- nor β,γ-unsaturated acids undergo anodic coupling (1,9,41,49,66,67, 78,80,110,113). However γ,δ-hexenoic acid (VII) on electrolysis gives the decadiene (VIII), among other products (41), and the

$$\underset{\text{(VII)}}{CH_3\cdot CH{:}CH\cdot(CH_2)_2\cdot CO_2H} \longrightarrow \underset{\text{(VIII)}}{CH_3\cdot CH{:}CH\cdot(CH_2)_4\cdot CH{:}CH\cdot CH_3}$$

γ,δ-unsaturated half ester (IX) couples normally to give the diester (X) (91). Unsaturated acids such as petroselinic (138), palmitoleic

$$\underset{\text{(IX)}}{EtO_2C\cdot CH{:}\overset{\overset{Me}{|}}{C}\cdot(CH_2)_2\cdot CO_2H} \longrightarrow \underset{\text{(X)}}{EtO_2C\cdot CH{:}\overset{\overset{Me}{|}}{C}\cdot(CH_2)_4\cdot\overset{\overset{Me}{|}}{C}{:}CH\cdot CO_2Et}$$

(17), oleic (15,16,25,81,110,138), ricinoleic (81), undec-10-enoic (15,63,110,116), and erucic (138), in which the double bond is even further removed from the carboxyl group, have also been used successfully in Kolbe syntheses. Moreover, model studies with oleic and elaidic acids have shown that the geometrical configuration of the double bond is preserved during coupling (15). Consequently, a definite configuration can again be assigned to the anodic product, if that of the starting material is known.

Like the corresponding ethylenic compounds, α,β-acetylenic acids do not undergo anodic coupling (141), but acids and half esters in which a triple bond is well removed from the carboxyl group take part in the Kolbe reaction normally (7,8).

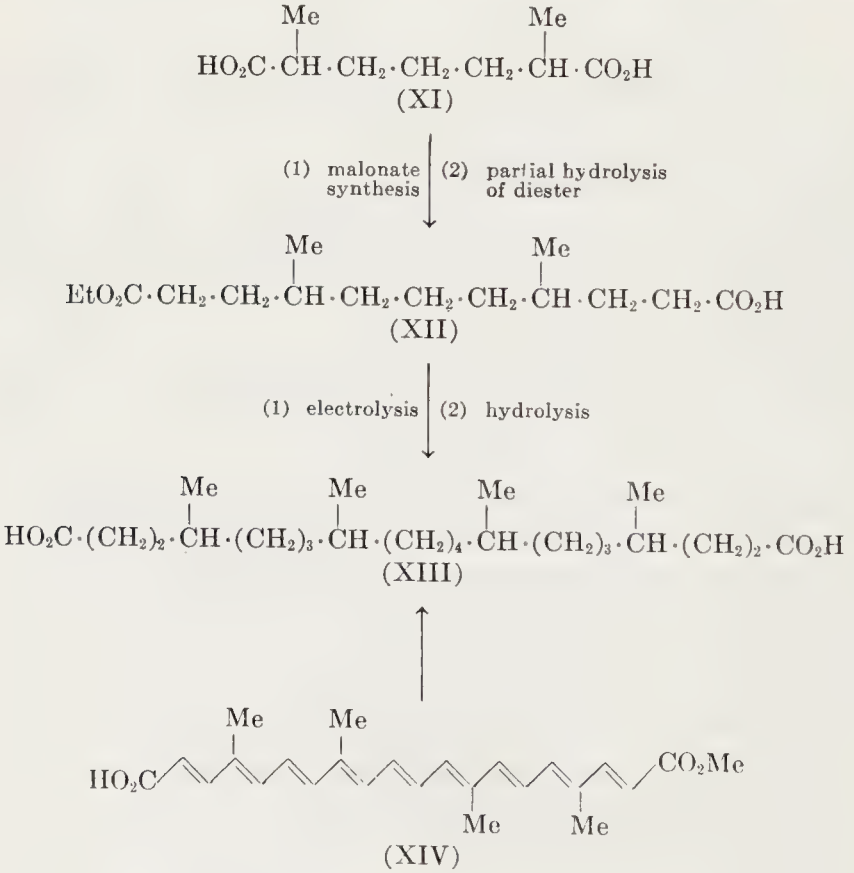
In view of the anodic behavior of α,β-ethylenic and acetylenic acids, it is not altogether unexpected that aromatic acids, in which the carboxyl group is directly attached to a benzene ring, also fail to undergo the Kolbe reaction to any significant extent under the usual conditions (48,53,54). This restriction does not apply to ω-phenylfatty acids (35,53,81,93; see, however, 19a).

III. Applications

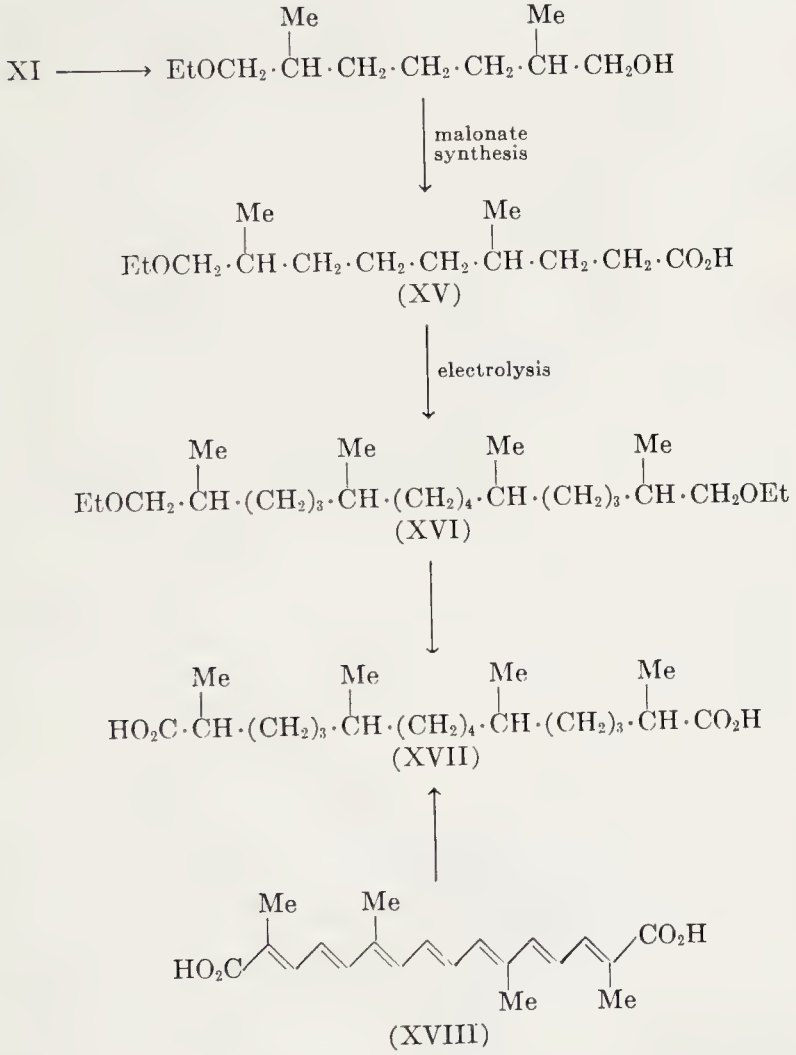
The pioneer work of Kolbe, of Wurtz, and of Brown and Walker, completed well before the turn of the last century, clearly indicated

the potentialities of anodic coupling reactions for organic synthesis. Nevertheless, apart from the preparation of hydrocarbons and diesters by the electrolysis of straight-chain fatty acids and half esters, there were few attempts to exploit reactions of the Kolbe type until comparatively recently. Since 1950, however, Kolbe reactions have been employed in the synthesis of over forty natural compounds, mostly lipids.

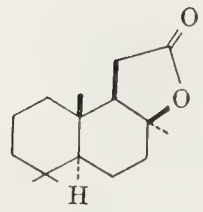
Two notable early uses of symmetrical coupling were in the preparation by Karrer *et al.* (76,77) of perhydrobixin (XIII) and perhydrocrocetin (XVII) in order to confirm the carbon skeletons attributed to the two carotenoids bixin (XIV) and crocetin (XVIII). The half ester (XII), prepared by a conventional malonate route from α,α'-dimethylpimelic acid (XI), was electrolyzed and the resulting diester hydrolyzed to give perhydrobixin (XIII). To achieve the second objective the same starting material (XI) was converted into the ethoxy acid (XV), which on electrolysis furnished the diether



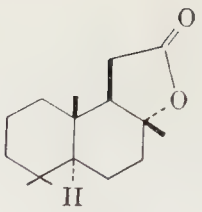
(XVI). The latter was transformed via the corresponding dibromide and glycol into perhydrocrocetin (XVII) by standard procedures. It was perhaps fortunate that in both instances the synthetic product, and that obtained by reduction of the natural carotenoid, apparently consisted of a similar mixture of the various possible racemates and yielded the same crystalline amide.



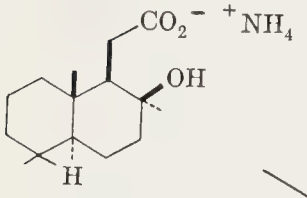
A most elegant use of symmetrical anodic coupling was in the recent total synthesis by Corey *et al.* (29) of pentacyclosqualene (XXII), a compound which had previously only been obtained by partial synthesis from α-onoceradienediol. The epimeric lactones (XIXa and XIXb) were converted into the corresponding γ-hydroxy acids from



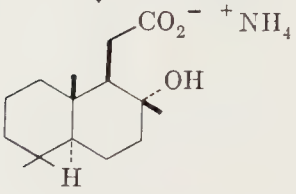
(XIXa)



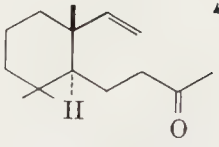
(XIXb)



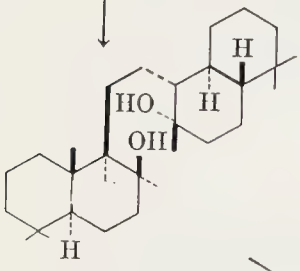
(XXa)



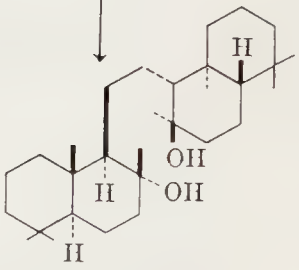
(XXb)



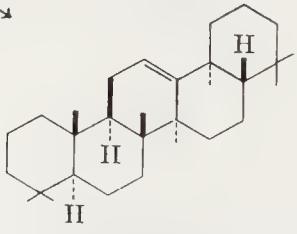
(XXIV)



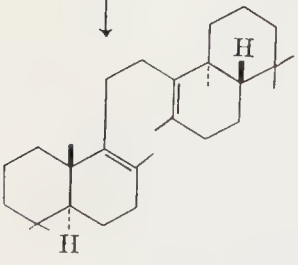
(XXIa)



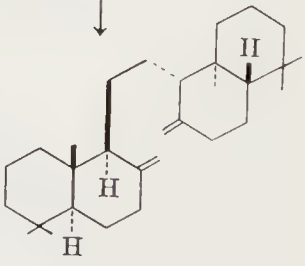
(XXIb)



(XXII)



(XXIIIa)

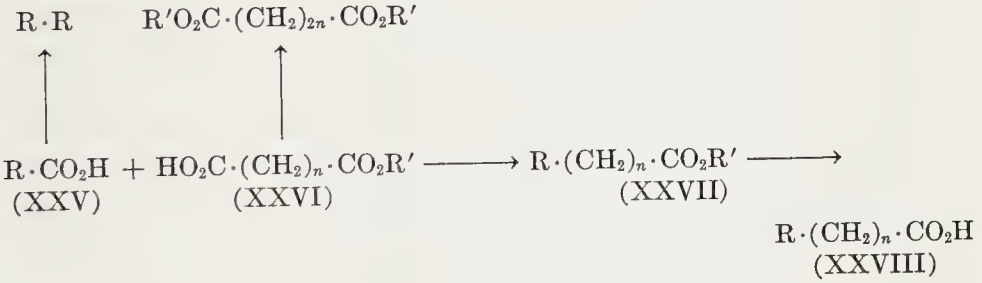


(XXIIIb)

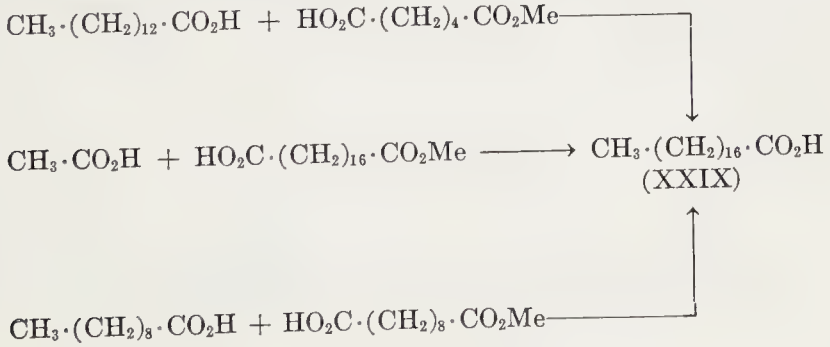
which the diols (XXIa and XXIb) were obtained by electrolytic coupling of the ammonium salts (XXa and XXb). Cyclization of either diol with perchloric acid in benzene–acetic acid then yielded (+)-pentacyclosqualene (XXII). By dehydration of the two diols (XXIa and XXIb) with phosphorus oxychloride–pyridine, syntheses of α- and β-onoceradiene (XXIIIa and XXIIIb) were also completed. These Kolbe reactions, which incidentally illustrate very well the retention during anodic coupling of configuration associated with both alkyl and hydroxyl substituents, were accompanied by a novel type of elimination reaction leading to the formation of the unsaturated ketone (XXIV). This competing process was avoided by acetylating the hydroxy acids prior to electrolysis (28).

A. SATURATED FATTY ACIDS

In 1895 von Miller and Hofer (143) introduced a method for the synthesis of fatty acids (XXVIII) which consisted in electrolyzing a mixture of a monocarboxylic acid (XXV) and a half ester (XXVI), and hydrolyzing the initial product of anodic crossed coupling (XXVII).



The original process has since been greatly improved (62), and now affords a convenient means of extending the chain of a carboxylic acid by any desired number of carbon atoms in virtually one operation.

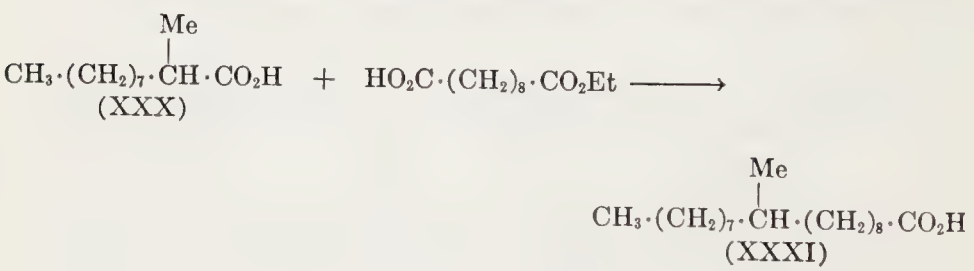


Stearic acid (XXIX), for example, has been prepared (62) by coupling myristic acid with adipic half ester, acetic acid with a half ester of octadecanedioic acid, and capric acid with sebacic half ester.

Apart from simplicity, the main advantage of the anodic route to fatty acids normally lies in the purity of the product. The initial mixture of hydrocarbon, mono ester and diester, formed by both symmetrical and unsymmetrical coupling of the two components, can often be separated readily by distillation. Alternatively, the mixture may be hydrolyzed, and the acid fraction separated into the dibasic acid and the required monobasic acid by distillation, crystallization, solvent extraction, or partition. By appropriate choice of starting materials, a product is obtained which is not contaminated with acids of similar physical properties, and is consequently easily purified. However, difficulty in purification is sometimes encountered with the products of small-scale anodic couplings involving chain extension by only a small number of carbon atoms. This arises mainly from contamination of the monoester formed in the desired crossed coupling reaction with small amounts of the methyl ester of the original acid. Such by-products are produced in side reactions of the starting material with the methanol normally employed as solvent during the electrolysis (see Section I). The difficulty in purification can be avoided by using benzyl in place of methyl or ethyl half esters. The benzyl ester resulting from crossed coupling can then be separated by distillation from any methyl ester of the starting material and subsequently hydrolyzed, or, alternatively, converted without isolation into the required acid by hydrogenolysis (33,95).

B. BRANCHED-CHAIN FATTY ACIDS

The electrolytic method is very suitable for the preparation of many branched-chain fatty acids, compounds of a type now known to occur in many natural lipids, though usually in only small amounts.



Asano and Ohta (4; cf. 101) synthesized the racemic form of tuberculostearic acid (XXXI) by coupling 2-methyldecanoic acid (XXX) with ethyl hydrogen sebacate. This successful crossed coupling of an α-substituted acid is interesting (see Section II), but the method cannot, of course, be used to prepare (−)-tuberculostearic acid, a major constituent of tubercle bacilli, and its dextrorotatory isomer. An alternative route which can be extended in this way was developed by Linstead, Lunt, and Weedon (88). Anodic crossed coupling of octanoic acid with methyl hydrogen β-methylglutarate (V) led to 3-methylundecanoic acid (XXXII). A further coupling with methyl

$$CH_3\cdot(CH_2)_6\cdot CO_2H + HO_2C\cdot CH_2\cdot \overset{\overset{Me}{|}}{\underset{*}{CH}}\cdot CH_2\cdot CO_2Me \longrightarrow$$

(V)

$$CH_3\cdot(CH_2)_7\cdot \overset{\overset{Me}{|}}{\underset{*}{CH}}\cdot CH_2\cdot CO_2H$$

(XXXII)

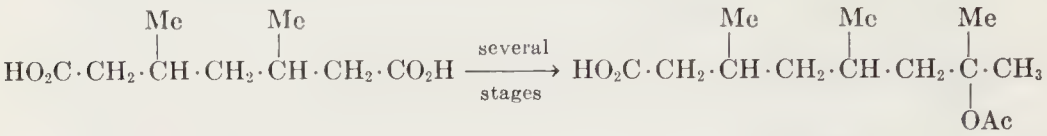
$$XXXII + HO_2C\cdot(CH_2)_7\cdot CO_2Me \longrightarrow CH_3\cdot(CH_2)_7\cdot \overset{\overset{Me}{|}}{\underset{*}{CH}}\cdot(CH_2)_8\cdot CO_2H$$

(XXXI)

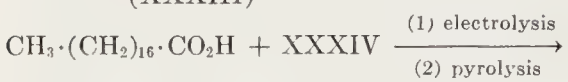
hydrogen azelate then yielded (±)-tuberculostearic acid (XXXI). When this synthesis was repeated using the readily available enantiomorphs of the half ester (V), no racemization occurred during either of the electrolytic stages and the pure (+)- and (−)-forms of tuberculostearic acid were obtained (90), the latter being identical with the natural material.

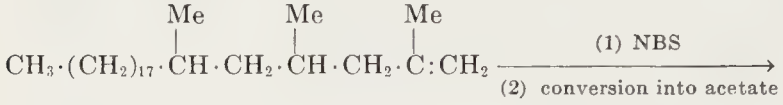
Anodic methods have also been used in some recent synthetic work on another branched-chain constituent of tubercle bacilli, mycolipenic acid, which is believed to be (+)-*erythro*-2,4,6-trimethyltetracos-*trans*-2-enoic acid. In one of a number of related syntheses of the methyl ester (XXXVII) of the racemic form of this acid, the acetoxy acid (XXXIV), prepared from *erythro*-β,β'-dimethylpimelic acid (XXXIII), was electrolyzed with stearic acid, and the initial product pyrolyzed, to give the olefin (XXXV). This was allowed to react with *N*-bromosuccinimide, and the resulting bromide converted into the acetate (XXXVI) from which the methyl ester (XXXVII) was obtained. Substitution in this synthesis of the ap-

propriate enantiomorph for the racemate of XXXIII should lead to mycolipenic acid itself.

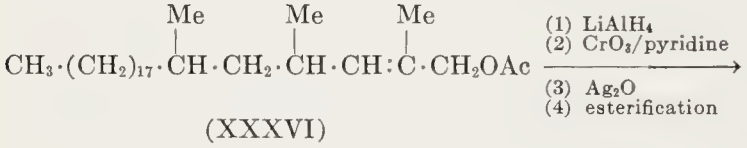


(XXXIII) (XXXIV)

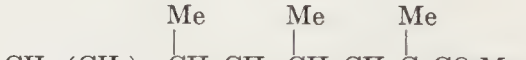




(XXXV)

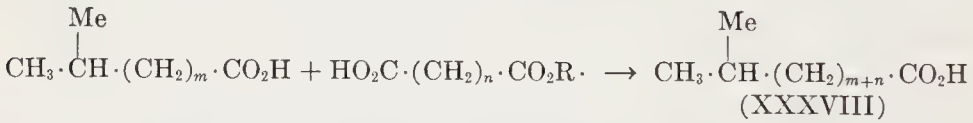


(XXXVI)



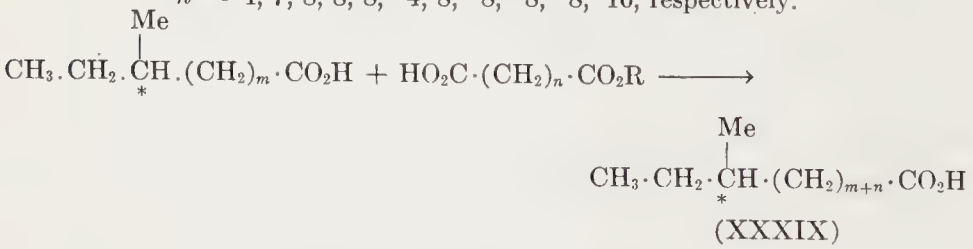
(XXXVII)

Other branched-chain lipids that have been synthesized anodically include the iso acids (XXXVIII) (72,96) and the dextrorotatory ante-iso acids (XXXIX) (96) which, together with the alcohols derived from them by reduction, are identical with important constituents of wool wax.



(XXXVIII)

m = 2, 1, 2, 4, 6, 10, 8, 10, 12, 14, 8 and
n = 4, 7, 8, 8, 8, 4, 8, 8, 8, 8, 16, respectively.

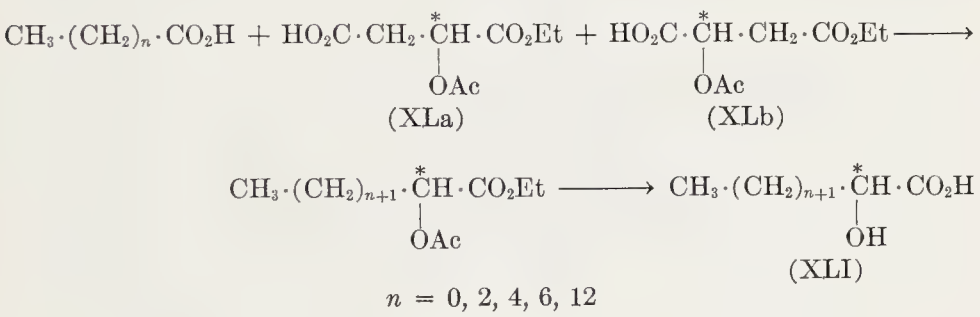


(XXXIX)

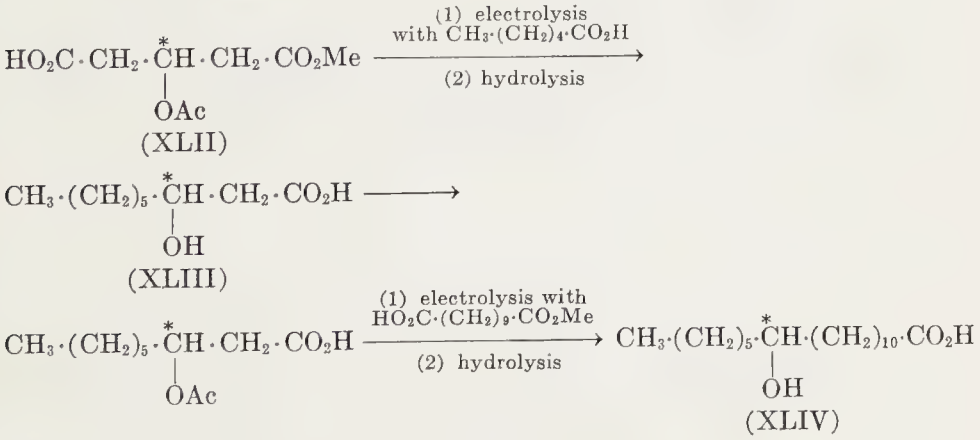
m = 2, 2, 1, 2, 4, 6, 8, 10, 4, 14, 18 and
n = 2, 4, 7, 8, 8, 8, 8, 8, 16, 8, 8, respectively.

C. HYDROXY COMPOUNDS

The α-hydroxy acids (XLI) form another class of acids found in wool wax and in other natural sources such as the cerebrosides. The stereochemistry of these compounds has been established by the anodic synthesis (71) of members of the opposite configurational series from fatty acids and the acetate of (−)-ethyl hydrogen malate (XLa). In this work advantage was taken of the fact that a component with an acetoxy substituent α to the carboxyl which is eliminated during electrolysis does not undergo coupling reactions of the Kolbe type (see Section II), and a mixture of the two positional isomers (XLa and XLb) of the half ester was actually used in the electrolyses.

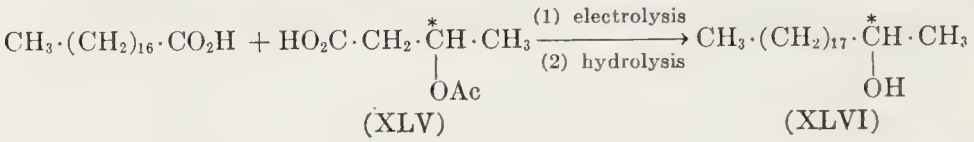


A more general route to optically active hydroxy acids is well illustrated by the recent determination (121; cf. 120) of the absolute configuration of ricinoleic acid. Anodic crossed coupling of hexanoic acid with (−)-methyl hydrogen β-acetoxyglutarate (XLII) led to (+)-3-hydroxynonanoic acid (XLIII), the antipode of an oxidative degradation product of methyl ricinoleate. Acetylation of XLIII.



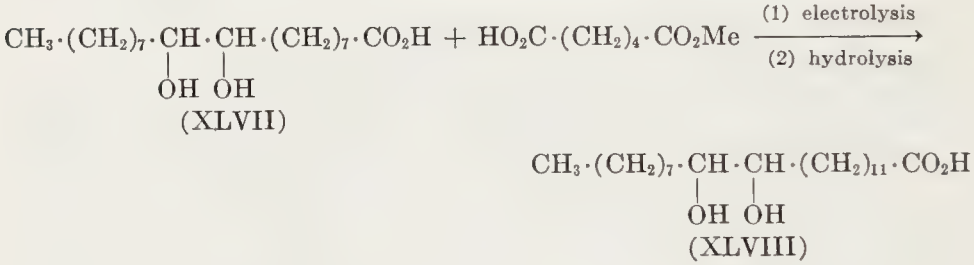
followed by an electrolysis with methyl hydrogen undecanedioate, then led to (+)-12-hydroxystearic acid (XLIV), the antipode of dihydroricinoleic acid. As the configuration of the starting material (XLII) is known, that of ricinoleic acid is now established.

The configuration of (+)-eicosan-2-ol (XLVI), an alcohol found in the waxes of the avian tubercle bacillus and the timothy bacillus, has been determined similarly. Hydrolysis of the acetyl derivative formed on electrolytic coupling of stearic acid with (+)-3-acetoxybutyric acid (XLV) gave a product identical with the natural alcohol (122). (For the synthesis of the terpene alcohol, phytol, see page 29.)



(XLV) (XLVI)

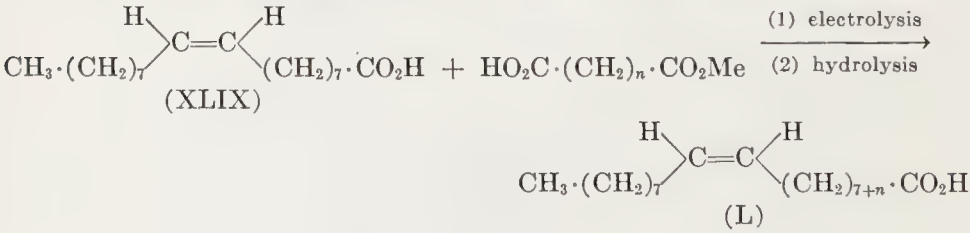
Both the *erythro* and *threo* forms of the (±)-dihydroxy derivatives of unsaturated fatty acids have been shown to undergo anodic coupling with retention of configuration (15,17). Electrolysis of a mixture of *erythro*-9,10-dihydroxystearic acid (XLVII) and adipic half ester gave *erythro*-dihydroxybehenic acid (XLVIII) (15).



(XLVII) (XLVIII)

D. UNSATURATED ACIDS

The anodic method has proved very convenient for the chain extension of unsaturated fatty acids. By crossed coupling with an appropriate half ester, oleic acid (XLIX), the most abundant fatty acid in

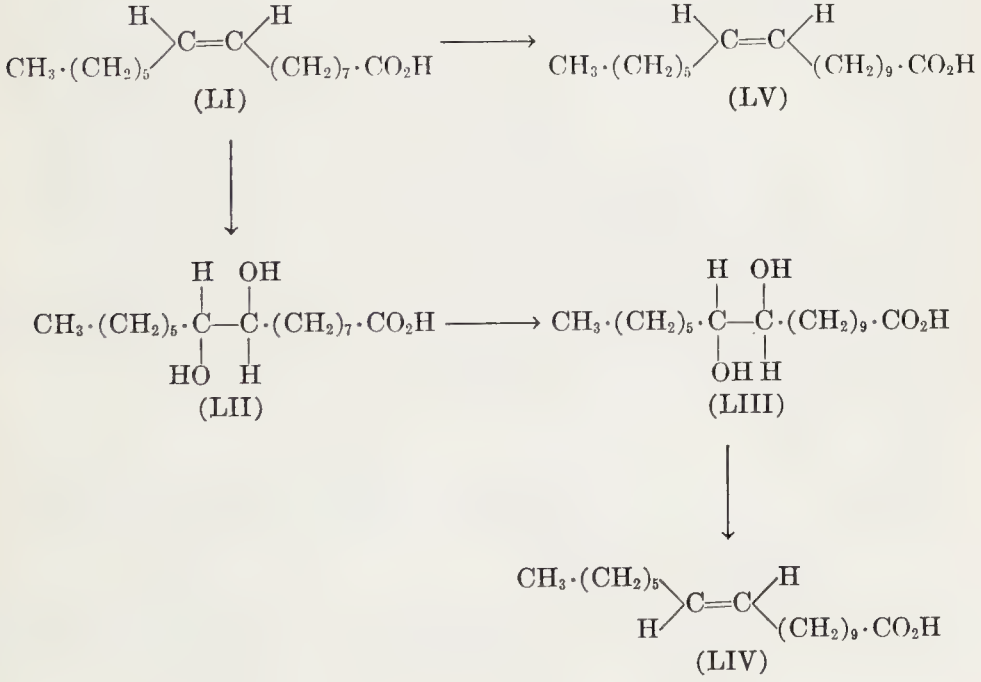


(XLIX) (L)

$n = 2, 4$, and 6

nature, has been converted into three comparatively rare acids, viz., eicos-11-enoic acid (L; $n = 2$) (6), a constituent of the unique liquid seed wax jojoba oil, erucic acid (L; $n = 4$) (15), a characteristic constituent of Cruciferae fats, and nervonic (selacholeic) acid (L; $n = 6$) (16), the principal unsaturated acid of the cerebrosides and a constituent of shark and ray liver oils. These syntheses established a direct stereochemical correlation between the products and oleic acid, and thus confirmed—or, in the case of eicos-11-enoic acid, provided the first proof—of their *cis* configurations. Substitution of elaidic acid (*trans*-XLIX) for oleic in the coupling reactions with adipic and suberic half esters led to the *trans* isomers of erucic and nervonic acids. No stereomutation of the double bonds was observed in any of these syntheses. The anodic method for the chain extension of unsaturated fatty acids is thus superior to the widely used malonate procedure, which not only involves more stages, but is apt to lead to partial stereomutation of the double bond (cf. 16,17). The anodic synthesis of 2-hydroxynervonic acid from erucic acid and the acetate of methyl hydrogen (+)-malate has also been claimed (71).

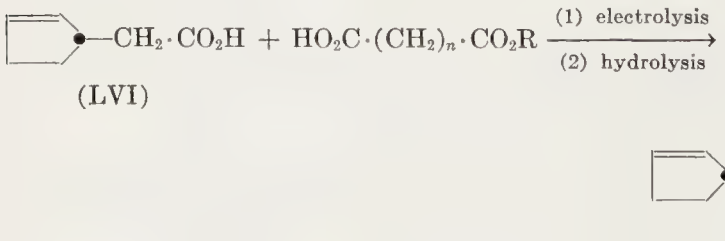
A way in which a *trans* product can be prepared from a *cis* starting material is illustrated (17) by a stereospecific synthesis of vaccenic acid (LIV), a trace constituent of many animal fats and the first



trans fatty acid to be isolated from nature. Performic acid hydroxylation of palmitoleic acid (LI), one of the main constituents of many marine animal oils, furnished *threo*-9,10-dihydroxypalmitic acid (LII) which was coupled anodically with methyl hydrogen succinate. The *threo*-11,12-dihydroxystearic acid (LIII) thus obtained was converted by conventional methods of bromination and debromination into the required *trans*-ethylenic acid (LIV). Direct chain extension of palmitoleic acid by electrolysis with methyl hydrogen succinate furnished the *cis* isomer (LV) of vaccenic acid which also occurs naturally; it is the principal unsaturated fatty acid of some bacteria and is identical with the hemolytic factor of horse brain.

Just as a *threo*-dihydroxy derivative of a fatty acid may be used to synthesize a *trans*-ethylenic acid, as in the above example, so the corresponding *erythro*-dihydroxy acid may be utilized in the preparation of the *cis* isomer of the ethylenic acid (15). These indirect anodic routes via dihydroxy intermediates do not normally afford any advantage over the direct chain extension of an unsaturated acid, except when it is desired to effect a stereomutation during the synthesis, or when either the starting material or the anodic product is difficult to purify. This latter contingency is most likely to arise in chain extensions with half esters of succinic and, presumably, malonic acid (cf. 17,95). A dihydroxy derivative then gives an anodic product which is more readily purified from traces of starting material (17,95).

Other examples of the direct chain extension of unsaturated acids are provided by the conversion of (+)-cyclopent-2-enylacetic acid (LVI) into chaulmoogric (LVII; $n = 11$) (98) and aleprestic (LVII; $n = 4$) (149) acids, two representatives of a class of cyclopentenyl acids found in the *Hydnocarpus* oils. As the stereochemistry of the starting material has been correlated with that of D-glyceraldehyde, these syntheses prove that the natural cyclopentenyl acids possess the configuration shown in the adjacent reaction scheme.



Another route to unsaturated acids is provided by the anodic chain extension of acetylenic acids and half esters. Coupling of stearolic acid (LVIII) with adipic half ester yielded behenolic acid (LIX) (8),

$$\underset{\text{(LVIII)}}{CH_3\cdot(CH_2)_7\cdot C{:}C\cdot(CH_2)_7\cdot CO_2H} + HO_2C\cdot(CH_2)_4\cdot CO_2Me \xrightarrow[\text{(2) hydrolysis}]{\text{(1) electrolysis}}$$

$$\underset{\text{(LIX)}}{CH_3\cdot(CH_2)_7\cdot C{:}C\cdot(CH_2)_{11}\cdot CO_2H} \xrightarrow{H_2/Pd\text{–}Pb} \underset{\text{(LX)}}{CH_3\cdot(CH_2)_7\overset{H}{\diagdown}C{=}C\overset{H}{\diagup}(CH_2)_{11}\cdot CO_2H}$$

while electrolysis of a mixture of octanoic acid and the acetylenic half ester (LXI) (7; cf. 30) led directly to tariric acid (LXII) (7), which occurs naturally in the seed fats of the genus *Picramnia*. Selective

$$CH_3\cdot(CH_2)_6\cdot CO_2H + \underset{\text{(LXI)}}{HO_2C\cdot(CH_2)_4\cdot C{:}C\cdot(CH_2)_4\cdot CO_2Me} \xrightarrow[\text{(2) hydrolysis}]{\text{(1) electrolysis}}$$

$$\underset{\text{(LXII)}}{CH_3\cdot(CH_2)_{10}\cdot C{:}C\cdot(CH_2)_4\cdot CO_2H} \xrightarrow{H_2/Pd\text{–}Pb} \underset{\text{(LXIII)}}{CH_3\cdot(CH_2)_{10}\overset{H}{\diagdown}C{=}C\overset{H}{\diagup}(CH_2)_4\cdot CO_2H}$$

reduction of the two acetylenic products over Lindlar's catalyst gave the corresponding *cis*-ethylenic acids, erucic (LX) and petroselenic (LXIII), which are characteristic constituents of the seed fats of the Cruciferae and Umbelliferae, respectively.

An extension (8) of the above reactions to a synthesis of oleic acid illustrates a more flexible route to unsaturated fatty acids than any so far mentioned. By coupling of the acetylenic half ester (LXV;

$$\underset{\text{(LXIV)}}{CH_3\cdot(CH_2)_{7-n}\cdot CO_2H} + \underset{\text{(LXV)}}{HO_2C\cdot(CH_2)_n\cdot C{:}C\cdot(CH_2)_n\cdot CO_2Me} \xrightarrow[\text{(2) hydrolysis}]{\text{(1) electrolysis}}$$

$$\underset{\text{(LXVI)}}{CH_3\cdot(CH_2)_7\cdot C{:}C\cdot(CH_9)_n\cdot CO_2H}$$

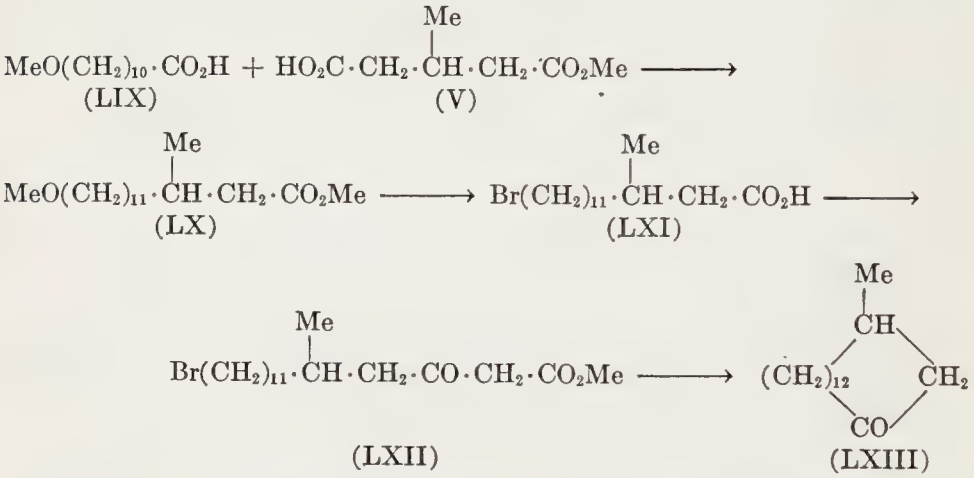
$$\text{LXVI} + \underset{\text{(LXVII)}}{HO_2C\cdot(CH_2)_{7-n}\cdot CO_2Me} \xrightarrow[\text{(2) hydrolysis}]{\text{(1) electrolysis}}$$

$$\underset{\text{(LVIII)}}{CH_3\cdot(CH_2)_7\cdot C{:}C\cdot(CH_2)_7\cdot CO_2H} \xrightarrow{H_2/Pd\text{–}Pb} \underset{\text{(XLIX)}}{CH_3\cdot(CH_2)_7\overset{H}{\diagdown}C{=}C\overset{H}{\diagup}(CH_2)_7\cdot CO_2H}$$

$n = 4$), or its lower homologue (LXV; $n = 3$), with a monobasic acid (LXIV), and then coupling the resulting acetylenic acid (LXVI) with a half ester (LXVII), stearolic acid (LVIII) was obtained. This on partial reduction over the Lindlar catalyst gave oleic acid (XLIX).

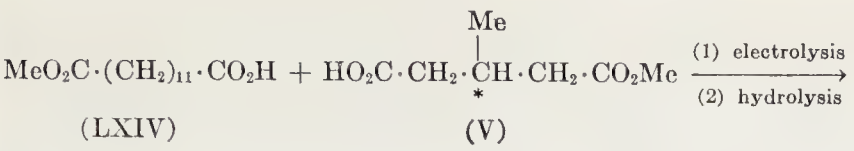
E. CYCLIC COMPOUNDS

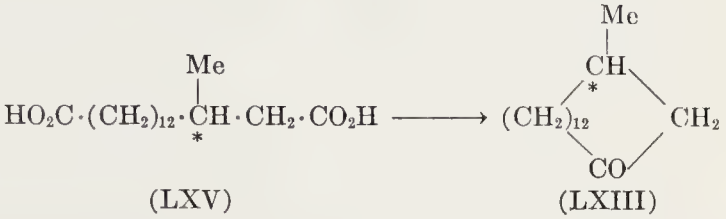
The synthesis of (±)-muscone by Hunsdiecker (75) was one of the first applications of the Kolbe reaction in the field of natural products. Electrolysis of a mixture of 10-methoxyundecanoic acid (LIX) and methyl hydrogen β-methylglutarate (V) led to the methoxy ester (LX) which was converted via the bromide (LXI) and the keto ester (LXII) into (±)-muscone (LXIII).



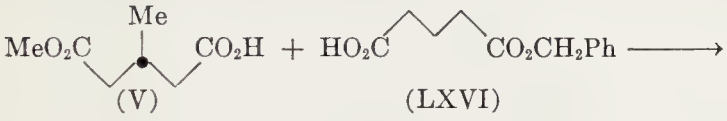
More recently the first syntheses of (+)- and (−)-muscone, the latter being identical with the natural material, were reported by Ställberg-Stenhagen (129). Electrolyses of mixtures of methyl hydrogen tridecanoate (LXIV) with the (+)- and (−)-forms of methyl hydrogen β-methylglutarate (V) yielded the two antipodes of β-methylthapsic acid (LXV). These were then converted via the diacid chlorides and the cyclic diketenes into (−)- and (+)-muscone (LXIII).

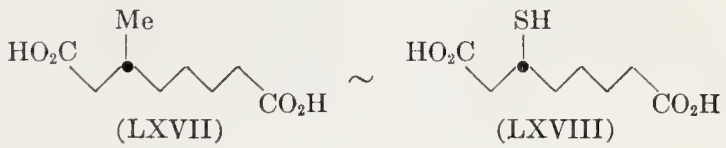
The method of establishing configuration by anodic synthesis has been adapted very ingeniously by Mislow and Meluch (97) to determine the stereochemistry of the cyclic disulfide (+)-α-lipoic (thioctic) acid. Benzyl hydrogen glutarate (LXVI) was coupled anodically with (+)-methyl hydrogen β-methylglutarate (V) to give, after

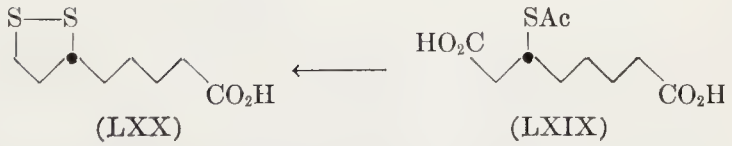




hydrolysis and hydrogenolysis of the initial product, (+)-3-methyloctanedioic acid (LXVII). This was shown to form a continuous series of solid solutions with (−)-3-thioloctanedioic acid, but to form a simple eutectic with the (+)-thiol. The (−)-thiol can therefore be assigned the same stereochemistry as (+)-3-methyloctanedioic acid, and hence as (+)-methyl hydrogen β-methylglutarate which has previously been correlated with D-glyceraldehyde. As the (−)-thiol



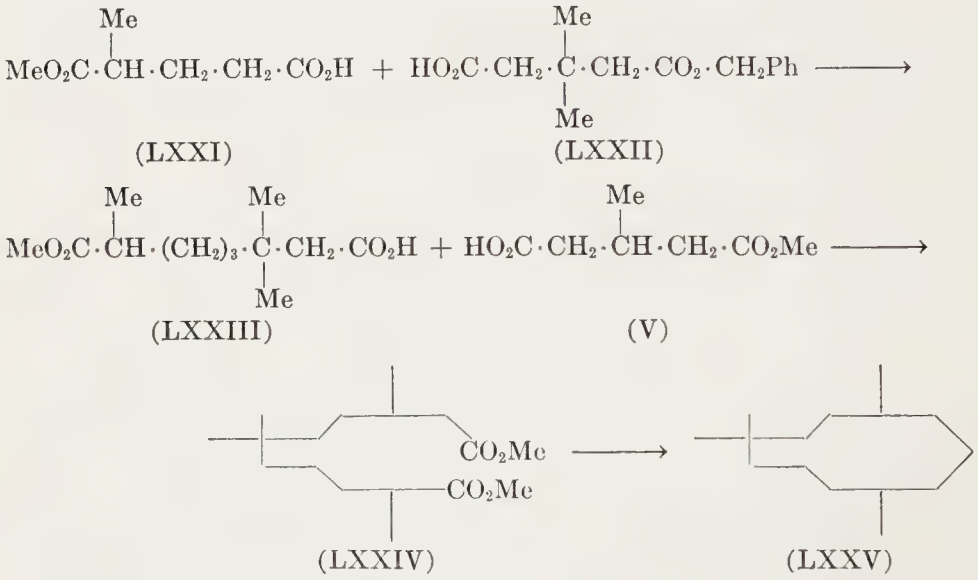




has been related via its *S*-acetate to (+)-α-lipoic acid, it follows that these three thio compounds must have the configurations shown in the accompanying reaction scheme as LXVIII, LXIX, and LXX, respectively.

The crossed coupling of a benzyl with a methyl half ester in the preceding example is noteworthy. This is a convenient procedure,

when the required unsymmetrical product would otherwise be rather similar to the symmetrical products, of increasing the differences and thus facilitating the separation. Subsequent hydrogenolysis of the benzyl group is also a valuable way of preparing an unsymmetrical methyl half ester for use in a further Kolbe reaction. Both these points are well illustrated in the method used by Šorm *et al.* (124) to confirm the carbon skeleton of the natural sesquiterpene humulene. Methyl hydrogen γ-methylglutarate (LXXI) was coupled anodically with benzyl hydrogen β,β-dimethylglutarate (LXXII), and the product hydrogenolyzed to give the unsymmetrical methyl half ester (LXXIII). The latter was then electrolyzed with methyl hydrogen β-methylglutarate (V), and the resulting unsymmetrical diester (LXXIV) was converted via the cyclic acyloin into the hydrocarbon (LXXV). This proved to be identical with humulane obtained by reduction of humulene.



IV. Experimental

The following notes are based on the electrolysis procedure normally employed in the author's laboratory.

Solvent. Commercial absolute methanol, containing traces of water, is usually the most satisfactory solvent.

Cells. For electrolyses on a laboratory scale, using up to about 250 g. of acids, large boiling tubes or cylindrical glass vessels are the

most suitable cells (Fig. 1). During the electrolysis the cell is placed in a cooling bath, usually of ice but occasionally of ice-salt or even alcohol–carbon dioxide. Large cells (e.g., 14 × 3 inches) are also fitted with internal cooling coils to help dissipate the heat generated. Owing to the vigorous evolution of carbon dioxide during electrolysis, no mechanical stirrer is necessary.

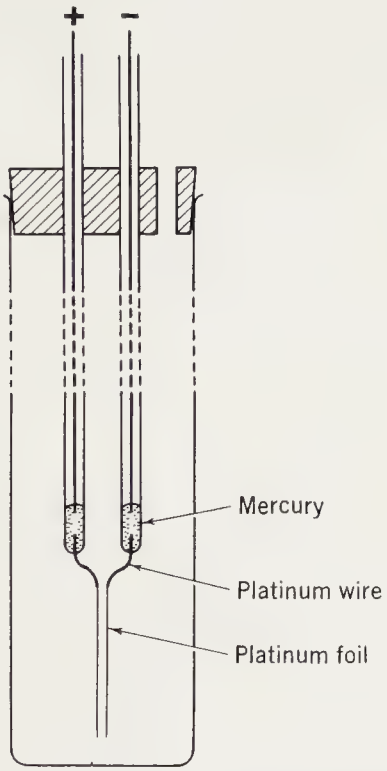


Fig. 1. Electrolytic cell.

Electrodes. A smooth platinum anode must be used (the only satisfactory alternative, iridium, is excluded on economic grounds). The nature of the cathode is less critical, but platinum is recommended so that the two electrodes can be made interchangeable, and the direction of the current reversed periodically. Cells are therefore usually fitted with two parallel electrodes cut from 0.002 inch platinum sheet. Each electrode is attached to a short piece of platinum wire by placing the two in contact, heating them in a blow-lamp flame until red hot, and then giving them a sharp blow with a hammer. The free end of the wire is sealed into a glass tube, great care being taken to

avoid leaving a capillary leak. Contact with the electrical circuit is made with small pools of mercury. Correct alignment of the electrodes about 2–3 mm. apart is simply achieved by gently pressing them between pads of filter papers. It is advisable to hang small glass rod spacers between electrodes greater than 4 × 4 cm. to prevent them being damaged by accidental contact during electrolysis.

Electrical circuit. A convenient circuit consists of a 110 volt d.c. supply, the cell, an ammeter (0–5 amp.), one or two large rheostats (about 50 ohms each), and a simple rocking commutator, wired as shown in Figure 2.

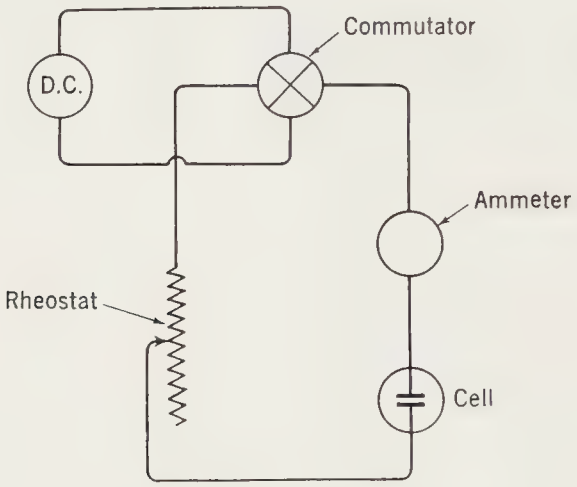


Fig. 2. Electrical circuit.

Procedure

Sufficient sodium is dissolved in methanol to neutralize about 2% of the acids to be used. The acids are added and the solution is electrolyzed until it is slightly alkaline. The number of Faradays required to complete the reaction is usually 1–1.5 times the theoretical for simple acids and methyl half esters, but may be about 3 times the theoretical for benzyl half esters.

During some electrolyses—in particular, with unsaturated acids—an insoluble deposit may form on one of the electrodes causing the current to drop. These deposits can often be dislodged by reversing the direction of the current from time to time by means of the commutator. In exceptional cases it may be necessary to interrupt the electrolysis at intervals and clean the electrodes.

Throughout the electrolysis, the temperature of the electrolyte is kept as near 20°C. as possible, and not allowed to rise above 50°C.

Some typical Kolbe syntheses are described below.

A. *n*-HEXACOSANE (87,147)

Myristic acid (5.0 g.) was added to a solution of sodium methoxide (from 0.1 g. of sodium) in methanol (25 cc.), and the solution was electrolyzed (current about 1 amp.) in a cylindrical glass cell (25 × 3 cm.) fitted with two platinum foil electrodes (3 × 2 cm., 2 mm. apart) until it was just alkaline. The cell contents were neutralized with a few drops of glacial acetic acid, and most of the solvent was then evaporated under reduced pressure. The residue was poured into water, and the mixture was extracted with ether. The ethereal solution was washed thoroughly with 2*N* sodium hydroxide, then with water, dried ($CaCl_2$), and evaporated. Crystallization of the residue from light petroleum (b.p. 40–60°) gave hexacosane (2.4 g., 60%), m.p. 57–58°.

B. METHYL β,β'-DIMETHYLSUBERATE (89)

Methyl hydrogen β-methylglutarate (35 g.) was added to a solution of sodium methoxide (from 0.57 g. of sodium) in methanol (90 cc.) and water (10 cc.). The resulting solution was electrolyzed (current 2 amp.) in a large boiling tube (2 inch diameter) fitted with a platinum foil cathode and a platinum spiral anode (from 20 cm. of 24 s.w.g. wire, surface area 3.52 sq. cm.). When the electrolyte became slightly alkaline (210 minutes), the electrolysis was interrupted. Concentrated sulfuric acid (1.22 g.) was added to the cell contents, which were then filtered and evaporated under reduced pressure until most of the solvent had been removed. Ether was added to the residue, and the ethereal solution was washed with sodium hydrogen carbonate solution, then with water, dried, and evaporated. Distillation of the residue gave methyl β,β'-dimethylsuberate (18.4 g., 80%), b.p. 101.5–102.5°/0.6 mm., $n_D^{18.5}$ 1.4376.

C. (+)-3-METHYLUNDECANOIC ACID (90)

A mixture of (+)-methyl hydrogen β-methylglutarate (10.0 g., 0.0625 mole) and octanoic acid (18.0 g., 0.125 mole) was added to sodium methoxide (from 0.09 g. of sodium) in methanol (80 cc.). The resulting solution was electrolyzed (current 2 amp.) in a cylin-

drical glass vessel fitted with two platinum foil electrodes (4 × 2.5 cm., about 1.5 mm. apart) until it was just alkaline. The cell contents from two such electrolyses were combined, neutralized by the addition of a few drops of acetic acid, and evaporated under reduced pressure. The residue was hydrolyzed with aqueous methanolic potassium hydroxide, and the products were separated into neutral and acidic fractions. Distillation of the neutral fraction gave *n*-tetradecane (12.0 g., 49%), b.p. 128°/19 mm., n_D^{21} 1.4275. Distillation of the acidic fraction yielded (+)-3-methylundecanoic acid (11.9 g., 48%), b.p. 117°/0.5 mm., n_D^{26} 1.4350, $[\alpha]_D^{23}$ + 4.50°. Crystallization of the residue (2.72 g.), m.p. 81.5–82.5°, from benzene–light petroleum (b.p. 60–80°), and finally from water containing a small amount of methanol, gave (+)-β,β'-dimethylsuberic acid (2.03 g.), m.p. 83–83.5°.

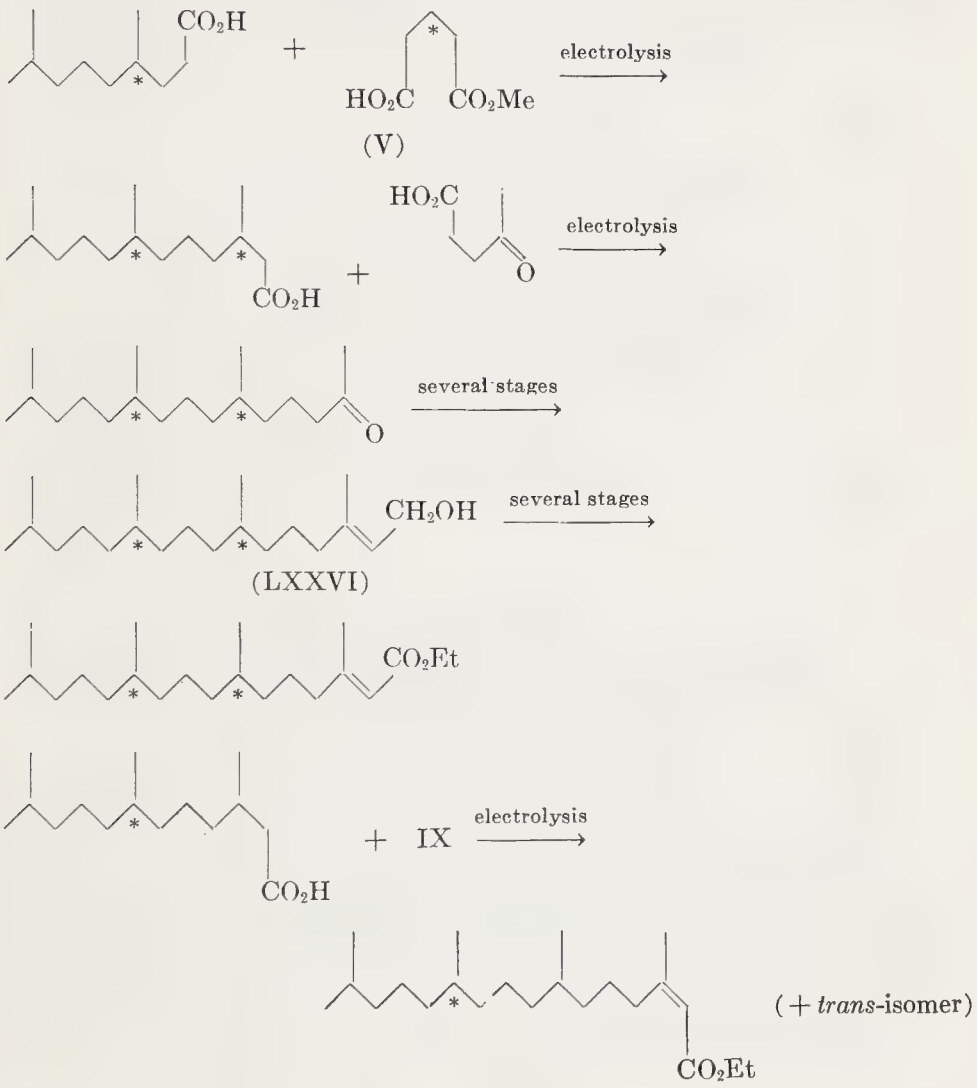
D. NERVONIC (*cis*-TETRACOS-15-ENOIC) ACID (16)

A mixture of oleic acid (10.0 g., 0.036 mole) and methyl hydrogen suberate (19.0 g.) was added to a solution of sodium methoxide (from 0.07 g. of sodium) in methanol (40 cc.), and the resulting solution was electrolyzed (current 1.4 amp.) in a cylindrical glass vessel fitted with two platinum foil electrodes (2 × 2.5 cm., 3 mm. apart), and two glass U-tubes through which cold water was passed. A colorless solid began to separate from the electrolyte at an early stage in the electrolysis. Towards the end of the reaction the current tended to drop, but was restored to its former value by the addition of further half ester (altogether 24.5 g., 0.13 mole, of the latter were used). After the electrolysis the alkaline cell contents were acidified and then evaporated. The residue was separated into acidic and neutral fractions. Distillation of the latter from a Kon flask gave: (*a*) an unsaturated oil (1.6 g.), b.p. 70–120°/10^{-5}mm., n_D^{19} 1.4409; (*b*) a product (10.5 g.), b.p. 127–130°/10^{-5}mm., m.p. 39–41°, which was not purified further (methyl tetradecanedioate has m.p. 43°); and (*c*) an oil (5.9 g.), b.p. 186–202°/10^{-5}mm., m.p. 12–13.5°, which was hydrolyzed with an excess of hot 2*N* aqueous methanolic sodium hydroxide and gave substantially pure nervonic acid (4.4 g., 37%), m.p. 37–39°. After regeneration from the lithium salt the product (3.0 g.) had m.p. 39.5–40.5°. Two crystallizations from alcohol at 0° yielded nervonic acid as plates, m.p. 40.5–41°.

The residue (0.9 g.) from the distillation consisted of crude tetratriaconta-*cis*-9-*cis*-25-diene.

Note Added in Proof

Anodic coupling reactions have recently played an important role in establishing the configuration of phytol (LXXVI) as 3,*D*-7,*D*-11, 15-tetramethylhexadec-*trans*-2-en-1-ol (25). The syntheses involved are summarized in the following reaction scheme:



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POLYPHOSPHORIC ACID AS A REAGENT IN ORGANIC CHEMISTRY

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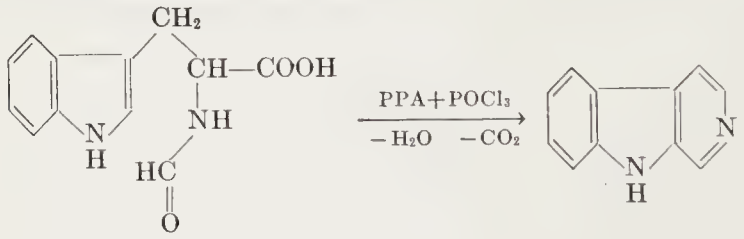
I. Introduction

In recent years the mixture of polyphosphoric acids ordinarily known simply as "polyphosphoric acid" (PPA) has found ever-increasing use in synthetic organic chemistry. First recognized as an exceptional reagent for cyclizations, it has since found application in various acid-catalyzed reactions, including esterifications, hydrolyses, condensations, acylations, rearrangements, and others. Prior to the recognition of PPA as a useful reagent in organic chemistry, there were scattered reports of the use of "mixtures" of phosphoric acid and phosphoric anhydride for the cyclization of aryl-substituted carboxylic acids to ketones. In the first such examples known to the authors (100,141) the anhydride was added to a mixture or solution of the organic material and the acid. In later applications, the two inorganic reagents were mixed, or the anhydride was dissolved in the acid, and the organic material was added (10,12,25,127). Since PPA can be prepared by heating phosphoric acid with the anhydride (Section II) it is quite likely that it was the active reagent in some of these

preparations. In more recent practice, either commercial PPA has been used or the mixture has been prepared by heating the acid and anhydride to permit their equilibration before use. For example, Gilmore and Horton (67) heated such mixtures on the steam bath for 2–4 hr. to prepare the reagent. After 2 hr. some solid material was still present, but evidently not in an amount sufficient to interfere in cyclizations of aryl-substituted acids. That unequilibrated mixtures of the acid and anhydride are not equivalent to PPA is indicated by the fact that attempts to effect the Sandmeyer isatin synthesis from isonitrosoacet-*p*-anisidide and mixtures of phosphoric acid and anhydride (146) were entirely unsuccessful, although later PPA was found suitable for the Sandmeyer cyclization (53) and, indeed, for the closure of the same anisidine derivative (128).

The first mention of a polyphosphoric acid as a catalyst for an organic reaction which the authors have seen is that of Rosen, who obtained a patent in 1941 for the condensation of an olefin with a branched paraffin over a catalyst containing "tetraphosphoric" acid (135). The use of tetraphosphoric acid for the dealkylation of phenols was patented in 1945 by Stillson and Fishel (158).

Despite these suggestions of the potential value of PPA in organic chemistry, the reagent first received serious consideration as a generally useful condensing agent in 1950 as the result of an accident. In an attempt to cyclize *N*-formyl-*dl*-tryptophan to 3,4-dihydro-*β*-carboline-3-carboxylic acid with phosphorus oxychloride, Werber (153) happened to use the latter reagent from a bottle which had been opened and allowed to stand imperfectly sealed in the laboratory for some years. Evidently not only did the desired cyclization occur, but also aromatization and decarboxylation to norharman (I),



(1)

(I)

the synthesis of which had been expected to require two additional steps. Attempts to duplicate the reaction with fresh phosphorus oxychloride and with various other acidic materials were unsuccessful until a mixture of commercial PPA and phosphorus oxychloride was tried. The success of this mixture led to the conclusion that PPA had

formed in the reagent bottle as a result of hydrolysis and polymerization, and a systematic study of the use of PPA was begun (154). Many useful applications were reported within a few years, and reviews of them shortly began to appear. Uhlig (168) first reviewed the literature on cyclizations with PPA in 1954. A short summary of applications in various reactions has been given by Kennard (96), and brief collections of data on the use of PPA in the acylation of phenols have been prepared by Baba (11) and Kusuda (106). A very valuable review of all the applications of PPA, extensively tabulated, was published by Popp and McEwen in 1958 (129). The present treatment is intended to cover only those applications in which PPA appears to have peculiar advantages, and not all the literature citations of the reagent will be considered. However, many of those omitted will be found in the article by Popp and McEwen (129).

II. Constitution of the Polyphosphoric Acids

So much has been written about the constitution of the "higher phosphoric acids" that it is difficult to give a brief summary of the entire subject. Accordingly, only the papers by Bell (21), Huhti and Gartaganis (89), and Thilo and Sauer (163) will be cited here. It can be stated that the thermal dehydration of phosphoric acid, the reaction of phosphorus oxychloride with phosphoric acid, and the reaction of phosphoric anhydride with phosphoric acid all give identical mixtures of polyphosphoric acids. The equilibrium among the various components depends only on the degree of hydration and not on the method of preparation. The mixtures are conveniently classified according to the percentages of phosphoric anhydride and water from which they could be derived, usually expressed simply as P_4O_{10} percentage or content. Mixtures containing up to 85% of the anhydride contain only linear polyphosphoric acids in addition to orthophosphoric and pyrophosphoric acids. At higher P_4O_{10} contents, cyclic and crosslinked molecules also are present. According to Thilo (162), heating completely hydrolyzed phosphorus oxychloride produces exactly the same PPA as is obtained by heating phosphoric acid. The chlorophosphoric acids, intermediate hydrolysis products, can be converted to the cyclic trimetaphosphoryl chloride or to chloropolyphosphoric acids, depending on the extent of the original hydrolysis. Upon further heating, the chloropolyphosphoric

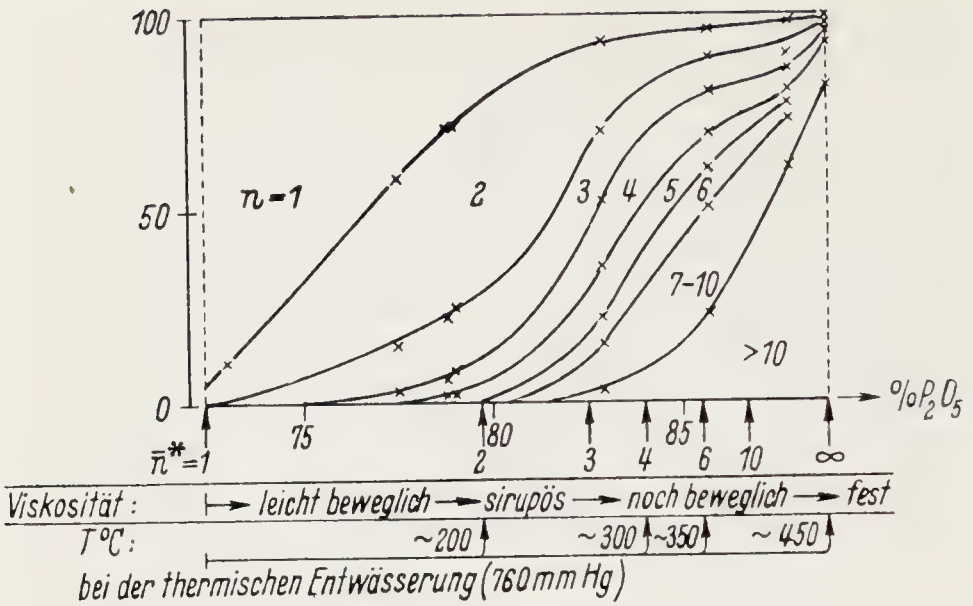


Fig. 1. Composition of polyphosphoric acids according to Thilo and Sauer (163). The figure shows the relation between the composition of polyphosphoric acid mixtures and the corresponding P_4O_{10} content. The intervals between the curves represent the percentage concentrations of the designated acids, n being the degree of condensation. The composition of any polyphosphoric acid mixture between 72 and 89% P_4O_{10} can readily be found by measuring, at any P_4O_{10} value on the abscissa, the vertical distances between the curves in terms of the percentage scale at the left. The values of $\bar{n}^*$ along the abscissa characterize the "average apparent degree of condensation," according to the general formula $H_{n+2}P_nO_{3n+1}$, as determined analytically. The temperature scale along the abscissa shows the temperatures at which the mixtures can be prepared by heating pure orthophosphoric acid, the concentration of which decreases with increasing P_4O_{10} content. (Reproduced by kind permission of Prof. Dr. Thilo)

acids yield crosslinked polymers, and these in turn can be equilibrated with water to the linear acids.

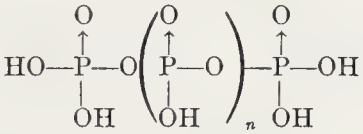
In Figure 1 are shown graphically the results of the careful study of the composition of the polyphosphoric acids carried out by Thilo and Sauer by paper chromatography.

Much of the work with PPA as a reagent in organic chemistry has been carried out with the commercial material available from the Victor Chemical Works, Chicago, Ill., and having a phosphoric anhydride equivalent of 82–84%. The composition of mixtures of approximately these percentages, as given by Huhti and Gartaganis

TABLE I
Composition of Two Polyphosphoric Acid Mixtures
(in per cent)

P_2O_5 equivalent	81.60	84.20
Orthophosphoric acid	8	4
Pyrophosphoric acid	27	11
Polyphosphoric acid		
$n = 1$	22	11
$n = 2$	17	13
$n = 3$	11	12
$n = 4$	6	10
$n = 5$	4	8
$n = 6$	2	6
$n = 7$	2	5
All higher values	1	20

(89), are shown in Table I. The value of n in the table is that in the formula



for the corresponding component; thus, $n = 1$ in tripolyphosphoric acid, 2 in tetrapolyphosphoric acid, etc.

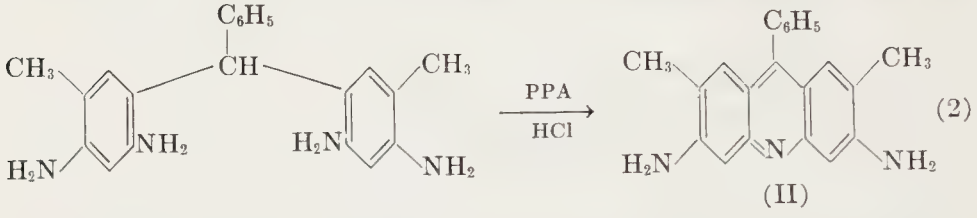
Since pure individual polyphosphoric acids are not obtainable by the methods employed, it is to be understood that in the sequel the term polyphosphoric acid is used to indicate mixtures similar to those shown in Table I; the term will be abbreviated as PPA.

III. General Character of the Reagent and of the Reactions Effected by It

The efficacy of PPA as a reagent in organic chemistry probably is the fortuitous result of a number of different properties of the mixture. It is a liquid which is a reasonably good solvent for organic compounds of many types, a fact that gives it a great advantage over its close relative, phosphoric anhydride. It contains both acidic and anhydride groups; water produced during the course of a reaction carried out in it is rapidly removed by reaction with the anhydride linkages, generating new acidic functions. Since water is a stronger base than many of the organic compounds involved in the reactions, its re-

moval in this manner may be of fundamental importance in preserving the effective acidity of the reaction mixture as a water-producing process occurs. As a dehydrating agent, PPA is much milder than phosphoric anhydride. As a result, there seldom is any difficulty with local overheating in a PPA reaction mixture. In fact, most of the reactions are carried out by heating the mixtures.

There are indications that the addition of hydrogen chloride, or of a substance which will react with PPA to generate hydrogen chloride, will in some instances change the character of the reagent, presumably as a result of increasing its effective acidity. Thus, Horning and Stromberg (82; see also Section IV-E) recommend the rearrangement of oxime *hydrochlorides* in PPA as a modification of the usual rearrangement of the oximes themselves. Similarly, Snyder and Konecky (151) found the preparation of benzoflavine (II) by the



(2)

(II)

simultaneous cyclization and aromatization of the corresponding tetramine hydrochloride to proceed in twice the yield obtained from the free amine (48% versus 24%). When the reaction was carried out with the free amine and sodium chloride was added to generate hydrogen chloride, the yield was intermediate (33%). All such reactions suffer the disadvantage that the evolution of hydrogen chloride causes the mixtures to foam. It would be of value to have a strong nonvolatile acid which would exert the same effect as hydrogen chloride without producing foam. *p*-Toluenesulfonic acid was ineffective in the benzoflavine synthesis, and, indeed, much of it was converted to the sulfone by the action of PPA (151).

The reactions which have been carried out in PPA are with few exceptions of types which can be effected with other acid catalysts or other dehydrating agents, although in numerous instances the use of PPA offers advantages in yield, ease of manipulation, or freedom from troublesome side reactions. In many synthetic processes the use of PPA permits the combination of two or more steps, so that the isolation of an intermediate is avoided. Thus, many cyclic ketones can be made directly from aryl-substituted acids or their esters as an

alternative to conversion to the acid chlorides and closure with aluminum chloride (Sections IV-A, B, C). Similarly, ketones can be subjected to the Beckmann rearrangement (Section IV-E) by treatment with a hydroxylamine source and PPA without the isolation of oximes, and acids and their derivatives can be subjected to the Lossen rearrangement without isolation of the hydroxamic acids (Section IV-F). A remarkable example of the formation of an oxime by reaction of hydroxylamine formed *in situ* is the conversion of fluorenone to its oxime by treatment at 190–200° with nitromethane in a solution of 1 part of P_4O_{10} in 2 parts of sirupy phosphoric acid (6). It was shown that nitromethane was converted to formic acid and hydroxylamine under essentially the same conditions. When, in the reaction with fluorenone, the temperature was raised to 250–255°, the oxime rearranged to phenanthridone; benzophenone gave the rearrangement product benzanilide (91%) at the lower temperature. It is to be noted that the acid mixture employed had a much lower phosphorus pentoxide equivalent than commercial PPA. It would be of interest to learn whether PPA would not convert a primary aliphatic nitro compound to the next lower primary amine by isomerization to the hydroxamic acid and Lossen rearrangement of the latter.

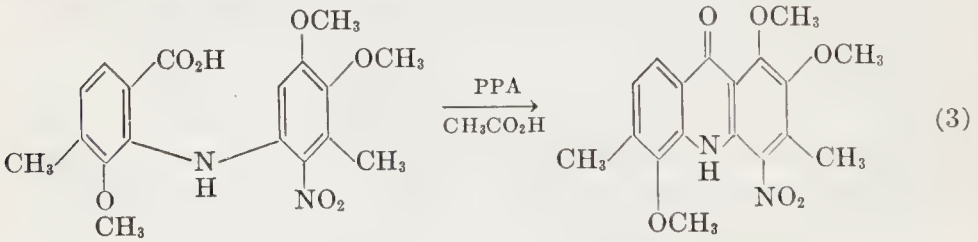
PPA is often used instead of a much more strongly acidic reagent such as aluminum chloride or concentrated sulfuric acid; as a result, molecules containing functions, such as ester groups, which are sensitive to the more vigorous reagents may be dealt with satisfactorily in PPA. The facts that PPA is not a strong oxidizing agent and has little or no tendency to enter into substitution on aromatic nuclei give it further advantages over concentrated sulfuric acid. As compared to hydrogen fluoride, particularly for cyclizing aryl-substituted carboxylic acids, it has advantages in that its handling requires no unusual precaution, so that simple apparatus can be used. In fact, as in the *Organic Syntheses* (155) preparation of α-tetralone from γ-phenylbutyric acid and PPA, a beaker and a stirring rod often suffice.

It should be mentioned that although PPA is not a difficult material to handle, it is not entirely innocuous. It will cause irritation of the skin, especially upon repeated contact; it is wise to wash affected parts immediately if exposure is suspected and to protect the eyes against accidental spattering of the material by wearing glasses or goggles when working with it. Since it is a viscous liquid,

it is less hazardous in the latter regard than many common reagents.

Rearrangements of carbon skeletons are less likely to be encountered in reactions carried out in PPA than in those effected by sulfuric acid, aluminum chloride, and other much more acidic reagents. Nevertheless, the possibility of rearrangement must be kept in mind, for some examples have been observed. Thus, in the cyclization of γ-2,4-dimethylphenylbutyric acid, Mosby (111) observed the formation of the ketone which would be expected if the butyric acid residue migrated from the 1-position of the ring to the 6-position before cyclization occurred (Section IV-B).

Experimental difficulties sometimes arise in the isolation of a product prepared in PPA as a result of the necessity of hydrolyzing the excess reagent and diluting it with water to the point where the solubility of the product is negligible. If the product is a base, it may also be necessary to neutralize the diluted phosphoric acid before the isolation can be effected. Since the reactions usually are carried out in amounts of PPA sufficient to serve as the solvent as well as the reagent, the quantities of aqueous solutions to be handled may become inconveniently large. Obviously, it would at times be advantageous to minimize the amount of PPA by using another substance as the solvent. Unfortunately, little work has been reported on the use of solvents. Brockmann and Muxfeldt (26) have used glacial acetic acid as the solvent in the cyclization of the heavily substituted *N*-arylanthranilic acid (III) to the acridone (IV), which was



(III) (IV) (3)

an intermediate in a synthesis of "despeptidoactinomycin." Even at room temperature undiluted PPA converted the anthranilic acid to resinous materials; by heating for 10 min. on a boiling water bath with a 15% solution of PPA in glacial acetic acid, a yield of 82% of the acridone was obtained. In this cyclization, the ring being acylated is highly activated by the four electron-donating substituents, despite the presence of the electron-withdrawing nitro group. In addition, the carboxyl group involved in the cyclization is located in a very

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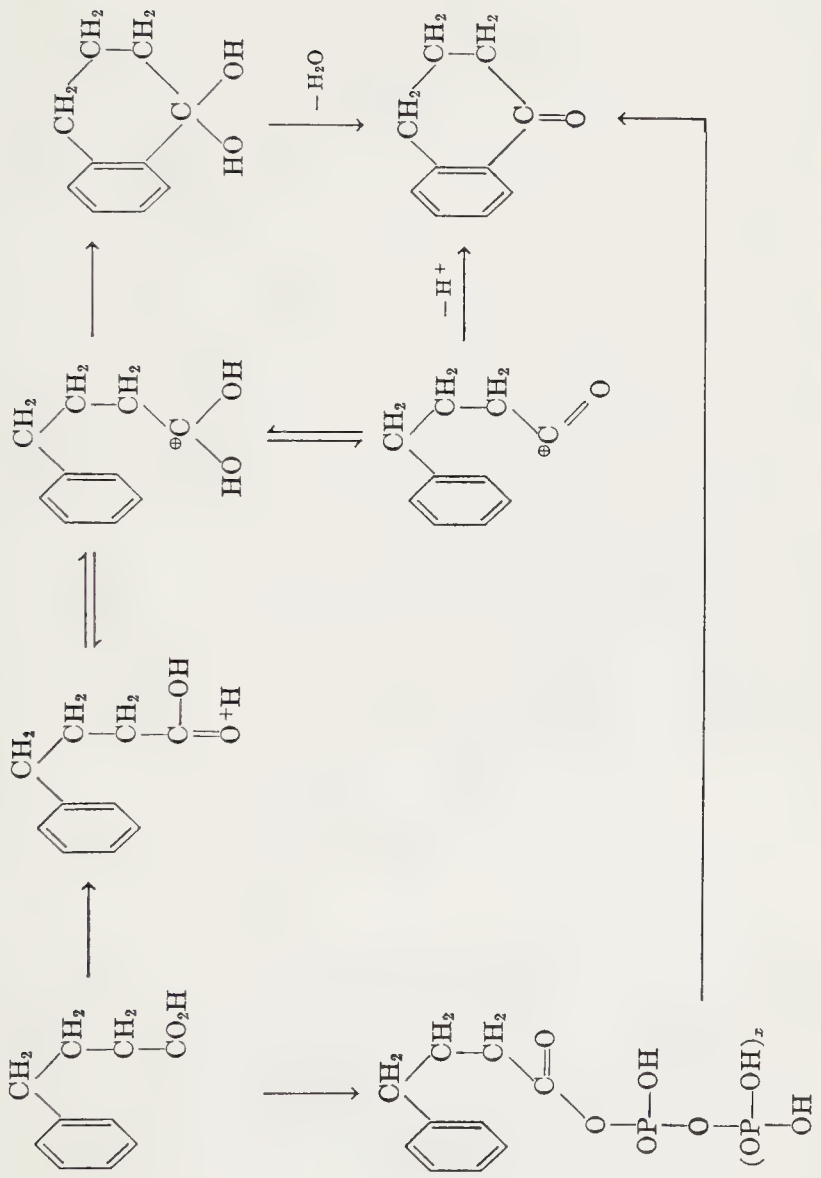
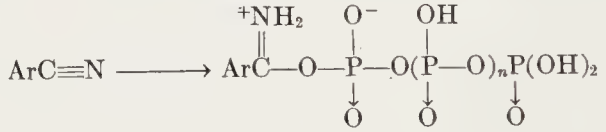


favorable position. It seems probable that only in such exceptional cases can carboxylic acids be expected to serve as useful solvents in intramolecular acylations, since they, too, are capable of acting as acylating agents in PPA (Section IV-D).

Since polyphosphoric acid is a complex mixture, it does not lend itself to the cryoscopic methods which have been so useful in the study of the mechanisms of the reactions of organic compounds in pure sulfuric acid (65). Because of its high viscosity, it is a poor medium for crystallization, and it is not surprising that no reaction intermediate has been isolated from it by this means. At the present time only surmises may be made concerning the mechanisms by which it operates on organic compounds. The most obvious possibilities are that it functions as a protonic acid, as a Lewis acid, and as a phosphorylating agent. Thus, the cyclization of γ-phenylbutyric acid may be rationalized on the basis of an initial step which is either protonation or conversion to a mixed anhydride as indicated in eq. 4. An oxocarbonium ion intermediate has been suggested for the conversion of γ-lactones to cyclopentenones (48).

The first step in the transformations of the Beckmann (Section IV-E) and Lossen (Section IV-F) types similarly may be the formation of phosphate esters of the —C=N—OH linkages. In reactions of nitriles, either in cyclizations (Sections IV-A, B, C) or in the conversion to amides (Section IV-F), it is possible that the first step is the addition to the triple linkage.



(5)

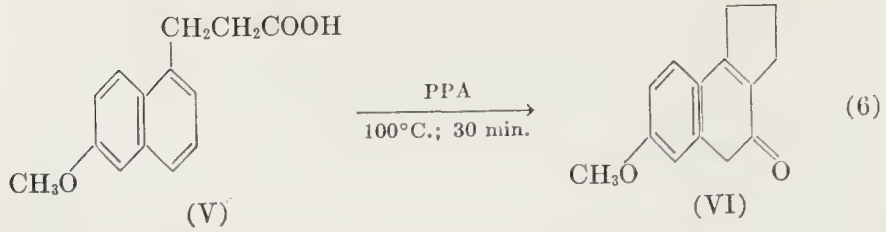
Such an intermediate might be expected to attack a neighboring aromatic residue in the same way as an acyl halide or an anhydride, leading to an imino ketone that is later hydrolyzed. Direct hydrolysis would lead to the amide. It is remarkable that the reagent can be used in the hydrolysis of nitriles to amides (Section IV-F), whereas its close relative, phosphoric anhydride, is a common reagent for the reverse transformation and 100% phosphoric acid is a common reagent for hydrolysis of nitriles to acids. Undoubtedly, the explanation lies in the formation of an intermediate, such as the one suggested above, which is stable in polyphosphoric acid but is rapidly hydrolyzed upon dilution of the solution with water.

IV. Principal Types of Reactions Effected by PPA

A. CYCLIZATION TO FIVE-MEMBERED RINGS

Many indanones have been prepared in high yields from the corresponding β-arylpropionic acids or their esters by warming with PPA (44,45,64,66,69,79,92,154). The examples include a number of methoxy-substituted indanones which could be obtained either only in low yields or not at all by the use of the classical reagents (25,79,80, 94,102,169). Self-condensation of the products under the influence of the PPA usually can be minimized by employing mild conditions (106,154). Elsner and Parker (52) rejected the reagent for the cyclization of certain β-tolylpropionic and β-tolylbutyric acids in favor of anhydrous hydrogen fluoride because of possible self-condensation and/or isomerization, but it is not clear whether their conclusion results from experience or is based only on analogy to a known isomerization in the tetralone series (Section IV-B). Although the question of isomerization in the synthesis of indanones from β-substituted acids higher than the propionic acids has not been extensively studied, rearrangement was not observed in the cyclization of β-phenylbutyric acid and its *m*- and *p*-methoxy derivatives (yields, 87, 86, and 17%, respectively), β-phenyl-β-methylbutyric acid (yield, 74%) and its *p*-methoxy derivative (yield 19%) (79), or of β-phenylvaleric acid (yield, 91%) (19).

Green and Hey (68) compared several reagents in the cyclization of thirteen 1-naphthyl-substituted aliphatic acids which carried methoxyl groups in the 4-, 5-, 6-, or 7-position of the ring. When the methoxyl group was in such a position that it activated the ring toward the cyclization, concentrated sulfuric acid proved suitable. When the methoxyl group was in other positions, sulfonation occurred when this reagent was used; with some such compounds PPA gave good yields of the ketones. α-Naphthylacetic acid, which gave only water-soluble products with sulfuric acid and only low yields in aluminum chloride-catalyzed cyclization of the acid chloride, gave a 40% yield of acenaphthenone with PPA. The 5- and 7-methoxynaphthyl derivatives gave the corresponding ketones in 50 and 30% yield, respectively, but the 4- and 6-derivatives gave no definite products. β-(6-Methoxy-1-naphthyl)propionic acid (V) underwent cyclization at the 2-position of the naphthalene nucleus, yielding the benzindanone (VI, 44%), which had been prepared earlier (24) and in somewhat better yield by the portion-wise addition of phosphoric anhydride to a mixture of the carboxylic acid and syrupy phosphoric acid.



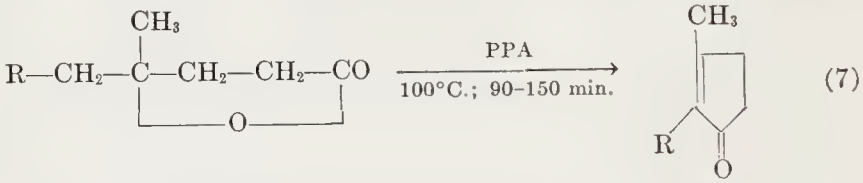
(V) (VI) (6)

When the methoxyl group was in the 5- or 7-position, cyclization into the *peri* position occurred, yielding the perinaphthenones (six-ring ketones).

Other cyclizations to benzofluorenones (92), 4,5-phenanthrylene ketone (136), and chromindanone (127) have been reported. Negative results with PPA were encountered in attempts to cyclize ethyl 1-β-naphthylmethyl-2-ketocyclohexanecarboxylate to a derivative of 3,4-benzofluorene which could be obtained in high yield with cold sulfuric acid (95), but 2-β-naphthylmethyl-1-tetralone gave an indene derivative which was dehydrogenated to 1,2,5,6-dibenzofluorene (23).

An attempt to cyclize β-3-indolylpropionic acid with "phosphoric oxide–orthophosphoric acid" failed, as did attempts with hydrogen fluoride and boron fluoride; phosphoric anhydride in xylene produced the expected ketone in 11% yield (93).

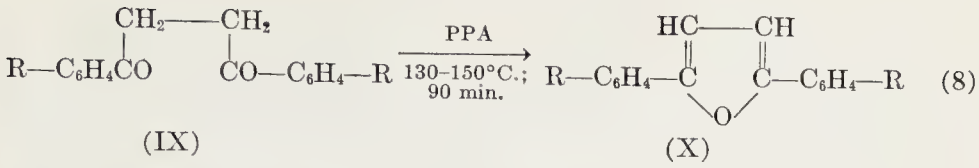
Purely aliphatic five-ring ketones (VIII) have been prepared by Rai and Dev (132) by the dehydration of γ-lactones (VII) with



(VII) (VIII) (7)

PPA. Yields above 90%, much better than obtained with other reagents, were not uncommon. Unsaturated ketones containing fused five and seven rings were made similarly from cycloheptenyl- and cycloheptylidenesuccinic acid derivatives (44,48,132).

For the closure of a number of five-membered heterocycles, PPA is the reagent of choice. Nowlin (123) first showed the value of PPA in such syntheses by preparing 2,5-diarylfurans (X) from 1,4-diketones (IX) in nearly quantitative yields. The reagent has been used by other authors for the preparation of benzofurans as well as dibenzofurans (41,44,165).



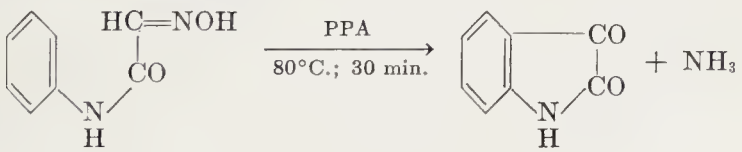
(IX) (X) (8)

A number of reports of the synthesis of indoles with the aid of PPA have appeared. The first applications were to the Fischer cyclization of arylhydrazones (53,98). The formation of the hydrazone derivative and its cyclization to the indole can be accomplished in a single operation. Thus, 2-phenylindole, 3-methyl-2-phenylindole, and 2-methylindole and 2-methyl-3-ethylindole were obtained directly from acetophenone, propiophenone, acetone, and diethylketone, respectively, in good yields. Chloroindoles have also been prepared with PPA (139). Parmerter, Cook, and Dixon (126) cyclized the *o*-, *m*-, and *p*-nitrophenylhydrazones of ethyl pyruvate by reaction with PPA at temperatures below 125° for 30 min., obtaining the ethyl nitroindolecarboxylates from which the simple nitroindoles were prepared by hydrolysis and decarboxylation. Earlier attempts to prepare 7-nitroindole by this method had failed because the *o*-nitrophenylhydrazone resisted the action of the usual catalysts (137); with PPA the yields from the *o*- and *p*-nitrophenylhydrazones were 57 and 66%, respectively, but, as expected, the *m*-nitro derivative closed in both the possible directions and only 19% of the 4-nitroindole ester and 8.2% of the 6-isomer could be isolated. In effecting the cyclization of the *o*-nitrophenylhydrazone at a higher temperature, Singer and Shive obtained only a 13.4% yield of the indole ester (143).

2-, 3-, and 4-Acetylpyridines were converted to the corresponding pyrindylindoles in good yields when their phenylhydrazones were heated with PPA at 180° for a few minutes (159). β-Carboline derivatives have been obtained by the Fischer cyclization of arylhydrazones of 3-keto-2-piperidone (2).

The preparation of 2,3-dimethylindole from *N*-butenylaniline and PPA has been patented (13).

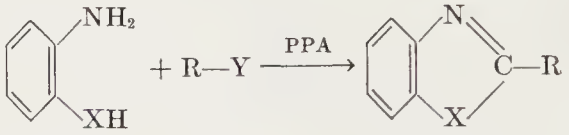
Isonitrosoacetanilide (XI) has been converted to isatin (XII) in 50–60% yields by the action of PPA (53,128). Derivatives with methyl



(XI) (XII) (9)

and methoxyl groups attached to the ring gave the isatins along with a little of the *N*-phenyloxamides. Derivatives with chlorine in the 2- and 4-positions gave 40–50% of the oxamides and little of the isatins. Nitro and amino derivatives gave only high-melting condensation products.

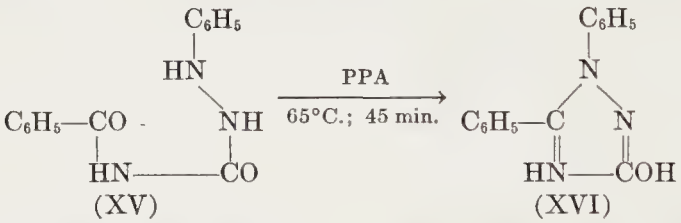
Hein, Alheim, and Leavitt (75) employed PPA in the preparation of 2-substituted benzimidazoles, benzoxazoles, and benzothiazoles (XIV) from acids, esters, amides, and nitriles in reactions with *o*-amino-, *o*-hydroxy-, and *o*-mercaptoanilines (XIII) (eq. 10). The yields were generally good, and the method has been extended to bisbenzimidazoles (173).



(XIII) (XIV) (10)

X = NH, O, S; Y = COOH, COOR, $CONH_2$, CN; R = alkyl, aryl

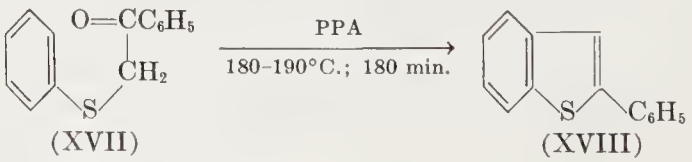
Derivatives of semicarbazide have been converted to substituted triazoles with PPA (9). Thus, 3-hydroxy-1,5-diphenyl-1,2,4-triazole (XVI) was obtained in 76% yield from 4-benzoyl-1-phenylsemicarbazide (XV). When the benzoyl group of the substituted



(XV) (XVI) (11)

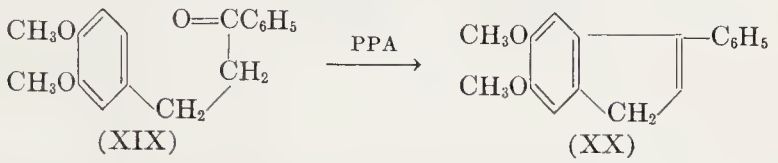
semicarbazide was replaced by a *p*-nitrobenzoyl group, the corresponding 1-nitrophenyltriazole was obtained, but the reaction failed with the 2,4-dinitrobenzoyl derivative.

In the synthesis of thianaphthenes by cyclization of α-arylthioketones, rearrangements have been observed. Whereas the benzoyl derivative (XVII) gave the rearrangement product (XVIII, 32%),



(XVII) (XVIII) (12)

the compound with an acetyl group in the place of the benzoyl group gave the unrearranged 3-alkylthianaphthenes (17,41). According to Daṇn and Kokorudz (39), when the reaction of XVII is effected at 100° for 2 hr., the isomeric 3-phenylthianaphthene is formed; hydrogen fluoride converts both XVII and 3-phenylthianaphthene to XVIII. Davies and Middleton (41) also found phenyl phenacyl ether to rearrange in the presence of PPA to yield 2-phenylbenzofuran. Rearrangement does not occur in the cyclization of the ketone (XIX) to the indene (XX) (41,102).

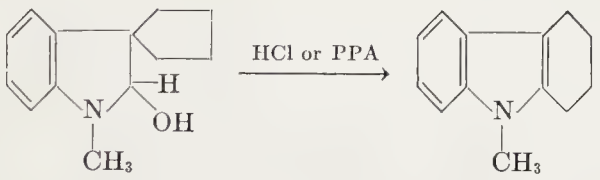


(XIX) (XX) (13)

Among other cyclizations to five-membered heterocycles with PPA may be mentioned preparations of thiadiazoles (107), oxadiazoles (53,129), arsafluorene oxides (28), thieno-[3,2-*b*]-pyrrole (110), and pteridines (167).

B. CYCLIZATION TO SIX-MEMBERED RINGS

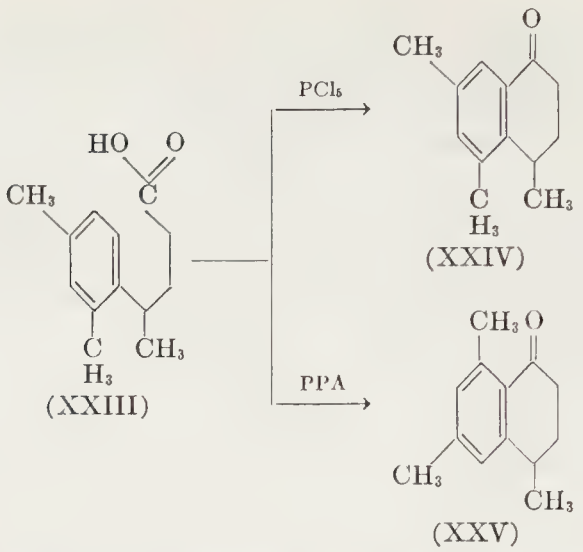
An interesting ring expansion, involving a rearrangement of the Wagner-Meerwein type, was discovered by Witkop and Patrick (175) in the conversion of the spirooxindole (XXI) to 9-methyltetrahydrocarbazole (XXII).



(XXI) (XXII) (14)

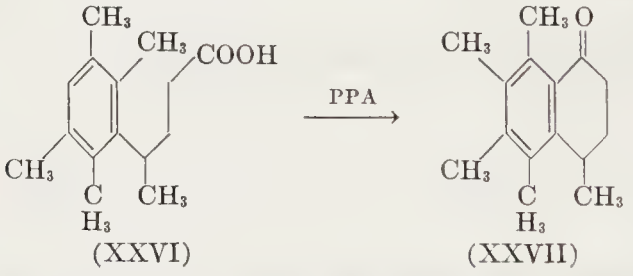
Most of the six-ring compounds that have been made with PPA are ketones obtained by cyclizing γ-arylbutyric acids. An example is the cyclization of γ-(2,4-dimethylphenyl)butyric acid to 5,7-dimethyl-1-tetralone in PPA at 150–170° for 2–3 min. with a yield of 83–90% (55). At higher temperatures 10,11-benzofluoranthrene was produced also.

Mosby (111) showed that rearrangement can occur in the PPA cyclization of substituted arylbutyric acids (eq. 15). Evidently the



(15)

butyric acid residue in XXIII moves to the symmetrical position before cyclization occurs, for heating of XXIV (prepared as shown with the aid of phosphorus pentachloride) did not effect its rearrangement to XXV. In the same work it was shown that methyl groups also can migrate, for γ-(2,3,5,6-tetramethylphenyl)butyric acid (XXVI) was cyclized by PPA to 4,5,6,7,8-pentamethyl-1-tetralone (XXVII) in 30% yield.

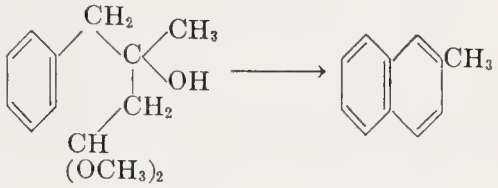


(16)

Nevertheless, a variety of tetralones have been prepared without difficulty due to rearrangement, and the method has been used especially for methoxy compounds (40,57,59,76,85,92,140,171). By employing benzylsuccinic acids, which might cyclize either to indanone or tetralone derivatives, Horning and Walker (84) showed that with PPA as with classical reagents of the Friedel-Crafts type, the formation of six-membered cyclic ketones occurs to the exclusion of the five-membered compounds. Methoxyl substitution on the aryl ring of the acid did not interfere with the PPA cyclization; such derivatives could not be cyclized with sulfuric acid.

A new synthesis of naphthalene derivatives was developed by Kotschetkov, Nifantyev and Nesmeyanov with the aid of PPA (105). Among the applications were the syntheses of 2-alkyl-, 2,6-dialkyl-, and 1,3,7-trialkylnaphthalenes. The synthesis of β-methylnaphthalene (XXVIII) is illustrative

$$CH_3COCH_2CH(OCH_3)_2 + C_6H_5CH_2MgCl \longrightarrow$$

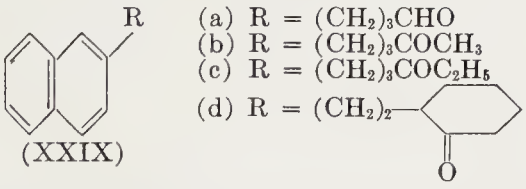


(XXVIII) (17)

Of the reagents examined for the cyclization-dehydration step, PPA and a mixture of 2 parts of phosphoric acid and 3 parts of sulfuric acid were found most effective. The acetals needed were made from chlorovinyl ketones.

The cyclization of *o*-benzoylbenzoic acids to anthraquinones with PPA requires rather more severe conditions than are needed for the preparation of tetralones. Anthraquinone (95% yield) itself has been prepared by heating *o*-benzoylbenzoic acid with PPA for 40 min. at 140° (154) and 1,2,3-trimethoxy-6,7-methylenedioxyanthraquinone (93%) and 1,2-benzanthraquinone (44%) by heating the corresponding acids at 80° for 6 hr. (102) and at 100° for 12 hr. (154), respectively.

A number of syntheses of polynuclear hydrocarbons, such as phenanthrene (7,12,22,42,50,100,119,122,124), chrysene (16,19,33), and xanthene (36), have been described. Djerassi and Pettit (50) investigated the PPA cyclization of a number of naphthalene derivatives, finding that γ-(β-naphthyl)butyraldehyde (XXIXa) was converted to phenanthrene, but that substituted ketones (XXIXb–d)



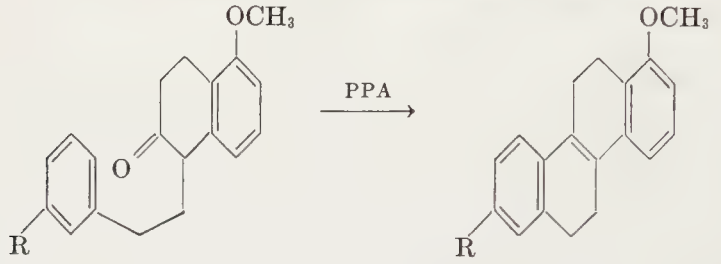
(XXIX)

(a) R = $(CH_2)_3CHO$
(b) R = $(CH_2)_3COCH_3$
(c) R = $(CH_2)_3COC_2H_5$
(d) R = $(CH_2)_2$—

gave anthracenes. Some of the phenanthrene derivative was detected in the products of cyclization of the methyl ketone (XXIXb),

but only the anthracene was isolated in the pure state. It was concluded that the steric interference between groups in the 4- and 5-positions of phenanthrenes is so great as to reverse the usual tendency toward the formation of angular rather than linear polynuclear hydrocarbons. The steric factor is not operative in the next lower homologs of XXIX, which undergo cyclization into the 1-position of the naphthalene nucleus to give indene derivatives.

Among the methods used for the synthesis of chrysene derivatives are the cyclization of 1,2,3,4-tetrahydro-2-phenyl-1-naphthylacetic acid by heating for 2 hr. at 120° with PPA (92), and the cyclization of methoxyketones (XXX) to methoxychrysene derivatives (XXXI).



(18)

(XXX)
(a) R = OCH_3
(b) R = H

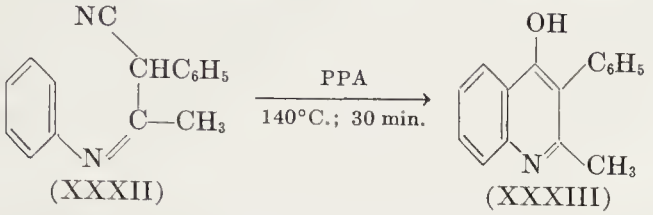
(XXXI)
(a) R = OCH_3 64%
(b) R = H 51%

PPA has been used in several applications of the Pechmann synthesis of coumarins. 4,7-Dimethylcoumarin was obtained from *m*-cresol and acetoacetic ester in 76% yield (53), and resorcinol has been condensed with acetoacetic ester, α-methylacetoacetic ester, and benzoylacetic ester to give the expected hydroxycoumarins in excellent yields (103).

PPA has been used for the synthesis of 4-chromanones, 4-thiachromanone, and 2,3-dihydro-1-aryl-4-(1*H*)-quinolines from β-aryloxypropionic acids, β-arylthiopropionic acids, and *N*,*N*-diaryl-β-alanines, respectively (91). When phosphorus oxychloride was used in some of these reactions, the products contained chlorine. A study of the mechanism of the Pechmann reaction by the conversion of β-arylcrotonic acids and β-keto esters to chromones and coumarins by reaction with phenols and PPA has been made (38).

Anils of β-ketonitriles and β-ketoamides have been converted to 4-hydroxyquinolines with PPA (74). 2-Methyl-3-phenyl-4-hydroxyquinoline (XXXIII), which could be obtained in only 4% yield by the method of Conrad and Limpach, was obtained in 56% over-all

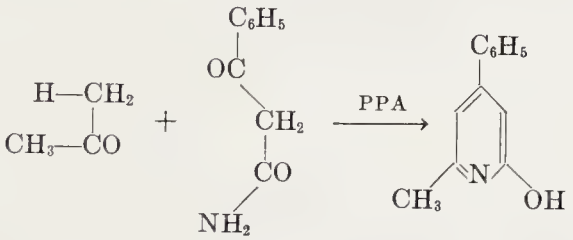
yield from aniline and α-aceto-α-tolunitrile by way of the anil (XXXII). The preparation of XXXIII from the nitrile and aniline



(XXXII) (XXXIII) (19)

in a single step was successful, but the yield was lower (39%). 2,3-Diphenyl-4-hydroxyquinoline was obtained similarly in 34% yield. 7,8-Dimethoxyisoquinoline has been obtained from the anil of 2,3-dimethoxybenzaldehyde and aminoacetal in a modification of the Pomeranz-Fritsch reaction with PPA at 100° as the cyclizing agent; yields of only 11–17% were obtained, but none of the product was formed when sulfuric acid was employed (49).

Hauser and Eby (73) have employed the reagent in a synthesis of substituted pyridones (e.g., XXXV) from β-ketonitriles and β-keto-



(XXXIV) (XXXV) (20)

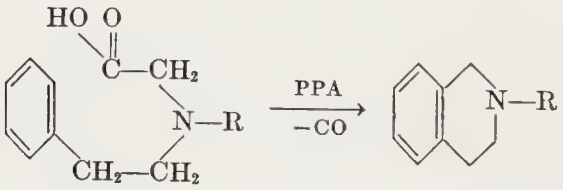
amides (XXXIV). The formation of a condensed pyridine ring occurred in the preparation of norharman by Snyder and Werber (Section I, eq. 1). Harman and 3,4-dihydroisoquinoline were also obtained from similar reactions in yields of 14 and 31%, respectively; the preparation of the latter compound has since been greatly improved (79% yield from β-phenylethylamine) by heating the crude formyl derivative with PPA at 160–180° for 2 hr. (130). The use of PPA in the synthesis of acridones (27) is referred to above (Section III).

Phenanthridine has been obtained in 90% yield from 2-formamidobiphenyl by vigorous stirring with PPA at 140° for 1 hr. (160); zinc chloride gave only a 42% yield, but a mixture of phosphorus oxychloride and stannic chloride in refluxing nitrobenzene (4 hr.) gave a yield of about 90% (125). A curious effect of agitation was noted

in the PPA-catalyzed preparation, for when the mixture was not stirred the only product isolated appeared to be *N,N'*-bis-(*o*-biphenylyl)formamidine (160). Brominated phenanthridones also have been prepared, but the PPA-catalyzed cyclization failed on 2-formamido-2-nitrobiphenyl and on 2,2'-diformamidobiphenyl. The last compound was doubly cyclized to the desired 4,9-diazapyrene with fused aluminum chloride–sodium chloride at 250–280° (8 hr.) (15).

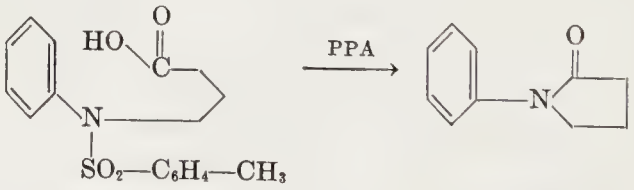
Other preparations of polynuclear heterocyclic systems which have been effected with PPA include 3,4-dihydro-4-phenylcarbostyril and -isocarbostyril (156), 4-(1,2,3,4-tetrahydro-1-isoquinolyl)hydrocarbostyril, the formation of which involved a rearrangement of heteroauxin-(β-phenethylamide) as well as cyclization (161), and a 2,4-dimethylindeno-(3',2':6,7)-quinoline from the anil of acetylacetone and 2-aminofluorene (29).

A novel preparation of a tetrahydroisoquinoline derivative has been described by Proctor and Thompson (131). When the *p*-tosyl derivative of *N*-β-phenethylalanine (XXXVI) was treated with PPA, water and carbon monoxide were eliminated and the tosyl derivative of tetrahydroisoquinoline (XXXVII) formed. However, the tosyl-



(XXXVI) (XXXVII) (21)

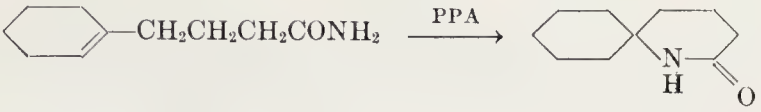
amide of *N*-phenyl-γ-aminobutyric acid (XXXVIII) gave 1-phenyl-2-pyrrolidone (XXXIX).



(XXXVIII) (XXXIX) (22)

Succinic acid and hydrazine hydrate in the molar proportions 2:1 gave perhydro-1,4,6,9-tetraketopyridazo-[1,2-α]-pyridazine in 80% yield under the influence of PPA (56).

A spiropiperidone (XLI) was obtained from γ-1-cyclohexenylbutyramide (XL) and PPA (77).

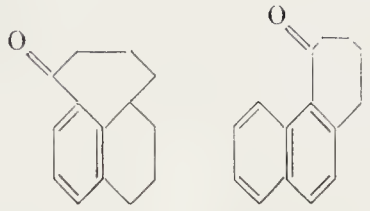


(23)

(XL) (XLI)

C. CYCLIZATION TO SEVEN-MEMBERED RINGS

PPA has been especially valuable in effecting the closure of seven-membered rings. Thus, benzosuberone has been prepared from δ-phenylvaleric acid in 84% yield by heating with PPA for 2 hr. at 100° (67,88), and in 90% yield from the methyl ester (66). Similarly, 7 - keto - 1,2,3,7,8,9,10,10a - octahydrocyclohepta - [de] - naphthalene



(XLII) (XLIII)

(XLII) has been made in 95% yield (88) from γ-1,2,3,4-tetrahydronaphthylbutyric acid, and the 1,2-naphthosuberone (XLIII) in 80–95% yield (164) from the corresponding acid. A methoxy derivative of XLIII is described in ref. 68. *o*-Phenylethylbenzoic acid gave an excellent yield of the expected dibenzocycloheptanone derivative (30).

Many substituted benzosuberones have been prepared by PPA-cyclization, and the reagent is especially useful on the methoxy substituted compounds. The preparations of methylbenzosuberones

TABLE II
Ring Closure to Methyl-Substituted Benzosuberones with PPA

Product	Time, min.	Temp., °C.	Yield, %	Ref.
1-Methylbenzosuberone-5	240	130–140	89	5
2-Methylbenzosuberone-5	2.5	161	88	55
2,3-Dimethylbenzosuberone-5	120	95	56	5
1,4-Dimethylbenzosuberone-5	35	95	67	5

are summarized in Table II and those of methoxybenzosuberones in Table III.

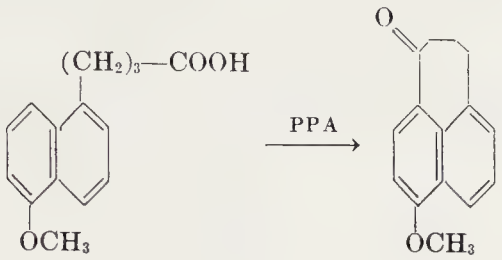
TABLE III
Ring Closure to Methoxy-Substituted Benzosuberones with PPA

Product	Time, min.	Temp., °C.	Yield, %	Ref.
1-Methoxybenzosuberone-5	30	95	—	63
1,2-Dimethoxybenzosuberone-5	30	95	67	63
2,3-Dimethoxybenzosuberone-5	30	95	74	63
2,3-Dimethoxybenzosuberone-5	60	75	84	102
1,4-Dimethoxybenzosuberone-5	55	60	55	5
1,2,3-Trimethoxybenzosuberone-5	50	70	91	102
2,3,4-Trimethoxybenzosuberone-5	30	95	99	63
2,3,4-Trimethoxybenzosuberone-5	40	100	91.2	62
2,3,4-Trimethoxybenzosuberone-5	20	100	79	62
2,3-Methylenedioxybenzosuberone	30	95	Polymer	63
2,3-Dimethoxybenzosuber-5-ene-6-carboxylic acid	30	10	66	101
Ethyl 2,3,4-trimethoxybenzosuber-5-ene-6-carboxylate	30	5	90	104
Ethyl 2,3,4-trimethoxybenzosuber-5-ene-5,6-dicarboxylate		5	97	104
2,3,4-Trimethoxybenzosuber-5-one-6-acetic acid (enol lactone)	11	100	60	62
2,3,4-Trimethoxybenzosuber-5-one-6-acetic acid (enol lactone)	11	100	98	62
Ethyl-2,3-dimethoxy-9-benzoyl-aminobenzosuber-5-ene-6-carboxylate	10	0–5	45	101

Other successful ring closures with PPA to a suberone of the phenanthrene series (88), to a ketohydrobenzazulene (3), and to a benzocyclohexanocycloheptanone derivative (4) have been reported. The reagent also has been used in the synthesis of colchicine intermediates (69).

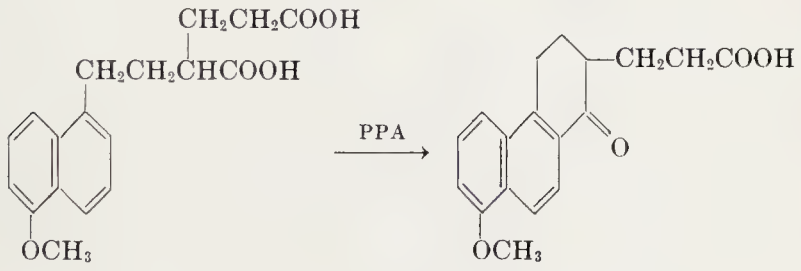
The reagent failed to effect the cyclization of γ-3,4,5-trimethoxyphenylpropylsuccinic anhydride; only with aluminum chloride in nitrobenzene was the benzosuberone derivative obtained (75% yield) (86). As indicated above, γ-(4-methoxy-1-naphthyl)butyric acid, and also δ-2-(4-methoxy-1-naphthyl)ethylglutaric acid cyclized to six-ring ketones (68). A methoxyl group in the 5-position of the

naphthalene ring bearing a carboxyalkyl side chain in the 1-position might be expected to promote closure into the *peri* position. The simple butyric acid derivative XLIV gave only the perinaphthanone XLV with PPA, sulfuric acid, or stannic chloride, in accord with this prediction. However, the glutaric acid (XLVI) gave a mixture from



(XLIV) (XLV) (24)

which only the ketophenanthrene derivative (XLVII) could be isolated. Similarly, α-1-naphthylmethylglutaric acid gave none of the perinaphthanone but only the indanone arising from closure into the 2-position (8).

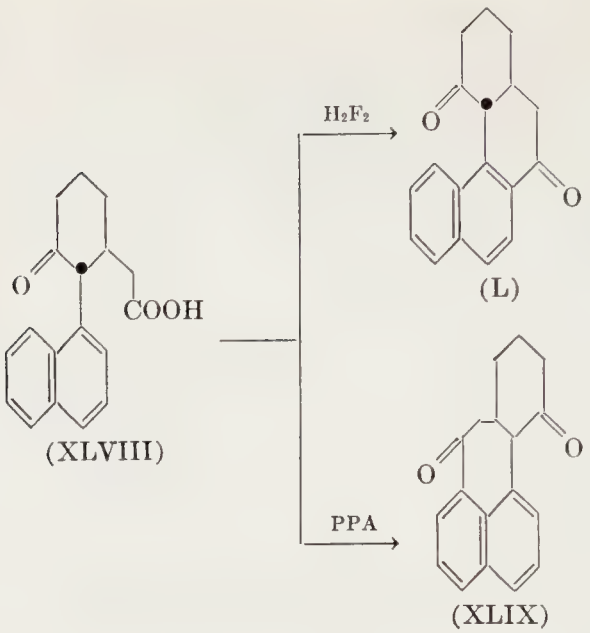


(XLVI) (XLVII) (25)

A diketone (XLIX) containing a seven-membered ring was prepared by Klibansky and Ginsburg (99) by treatment of the keto acid (XLVIII) with PPA. Hydrogen fluoride caused the alternative cyclization, yielding L.

An attempt to prepare a seven-membered ring by the action of PPA on the ketone LI failed (70). Instead of the expected product (LII), a mixture containing cyclohexylnaphthalenes and -tetralins was obtained. The original article may be consulted for a discussion of the rearrangements involved.

Stetter, Schäfer, and Spangenberger (157) studied the cyclization of acids of the type LIII, in which intermolecular condensation is strongly hindered sterically so that cyclization without recourse to



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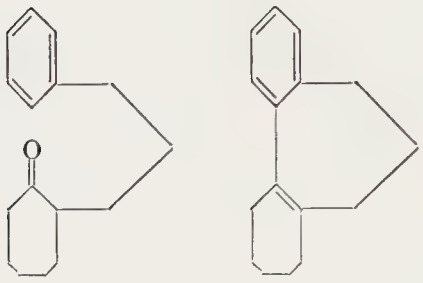
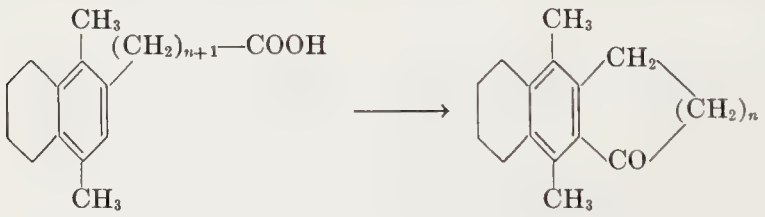


(LI) (LII)

high dilution might be expected to occur. The six- and seven-ring ketones (LIV, $n = 2$ and $n = 3$) were obtained in 75 and 80%



(LIII) (LIV)

(27)

yields, respectively, with PPA, but the next member (LIV, $n = 4$) could be prepared only by the use of aluminum chloride on the acid

chloride (49%). The nine-membered ketone could not be synthesized.

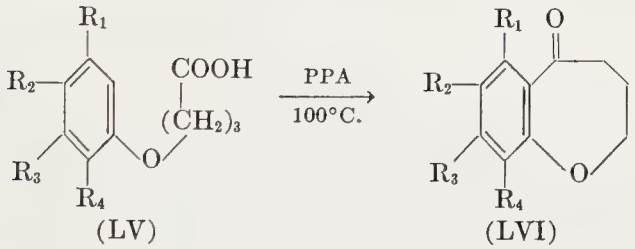
In the cyclization of γ-1-acenaphthylbutyric acid to the ketone containing a seven-membered ring, a PPA mixture containing 78% of phosphoric anhydride gave better yields than one containing 83% (133). The results appear in Table IV.

TABLE IV
Cyclization of γ-1-Acenaphthylbutyric Acid with PPA under Different Conditions

P_4O_{10}/acid, by wt.	P_4O_{10} in PPA, %	Time, min.	Temp., °C.	Yield, %
20/1	83	75	75	38.2
25/1	78	140	100	57.1
30/1	78	45	100	82.6
30/1	78	40	100	86.3

δ-Ferrocenylvaleric acid was cyclized with either PPA or trifluoracetic acid to the homoannular ketone, 1,2-(α-ketopentamethylene)-ferrocene (134). The γ-butyric acid closed similarly, but the β-ferrocenylpropionic acid underwent cyclization into the second cyclopentadienyl ring, giving the heteroannular ketone. A biscarboxypropylferrocene underwent double cyclization (121).

Homochromanone (LVIa) and its 7,8-dimethyl derivative (LVIb) have been prepared by PPA cyclization of γ-aryloxybutyric acids (37) in yields of 45 and 66%, respectively. The 8-methoxy com-



(LV) (LVI) (28)

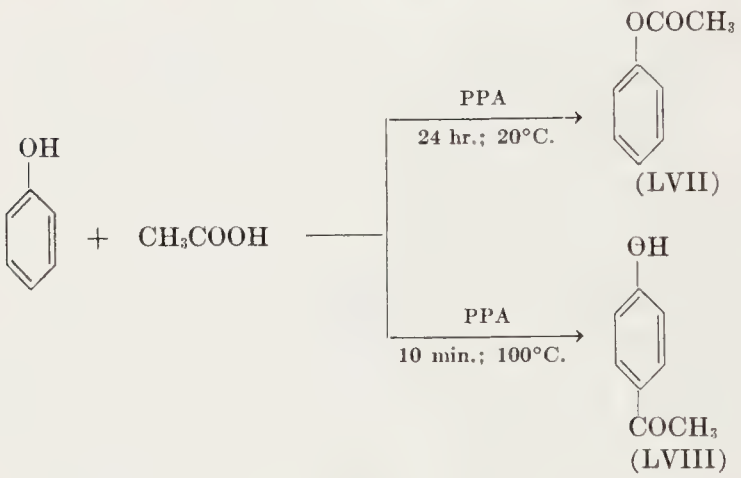
(a) $R_{1,2,3,4}$ = H
(b) $R_{2,3}$ = CH_3; $R_{1,4}$ = H
(c) R_3 = OCH_3; $R_{1,2,4}$ = H
(d) $R_{1,4}$ = CH_3; $R_{2,3}$ = H

pound (LVIc) and the 6,9-dimethyl derivative (LVId) could not be obtained by this method.

D. ACYLATION

PPA has been used in many intermolecular acylations as well as in the intramolecular reactions of the kind discussed above. The reaction of certain aromatic hydrocarbons with simple carboxylic acids was first reported in 1950 (106) to give crystalline hydrocarbons. The products from mesitylene and acetic, propionic, and butyric acids were later (152) found to be 1,1-dimesitylethylene, 1,1-dimesitylpropene, and 1,1-dimesitylbutene, produced in 9, 39, and 48% yields, respectively, by reaction at 140° for 3–5 hr. Chloroacetic acid gave mono- and dichloroacetylmesitylene. The formation of the dimesitylalkenes was attributed to further reaction of the acylmesitylene first formed with a second molecule of mesitylene.

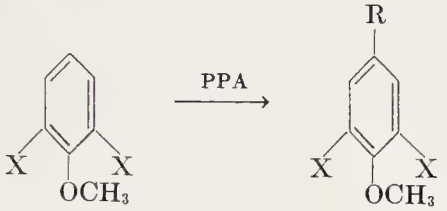
In 1953 the first reports of Nakazawa and his associates on the acylation of phenols with acids and PPA appeared (115), describing the preparation of both esters (LVII) and hydroxyketones (LVIII), depending on the conditions. The ester (LVII) was formed when



(29)

the reaction was carried out in the cold for 24 hr., and the ketone when the mixture was heated on a boiling water bath for 10–30 min. Similarly, resorcinol and acetic acid gave the diacetate, and the diketone gave 4,6-diacetylresorcinol; hydroquinone, phenolphthalein, and diphenolisatin gave the expected products with various aliphatic acids. Phenol and benzoic acid gave only the ester, however. These observations were extended in the following papers (117) and phenol, anisole, and catechol dimethyl ether were converted to ketones by reaction with aliphatic acids and cinnamic acid in hot PPA. Aro-

matic acids, including benzoic, salicylic, *p*-hydroxybenzoic, and anisic, usually gave esters. However, by reaction in the cold, phenol gave the *p*-hydroxyketones with benzoic acid, anisic acid, and *p*-hydroxybenzoic acid. Phenyl anisate could be rearranged to the hydroxyketone in PPA, but not phenyl benzoate. Gardner (60), in an independent investigation of the reaction, condensed anisole and pyrogallol trimethyl ether with acetic acid, acetic anhydride, benzoic acid, and methyl hydrogen succinate to the corresponding ketones (LIX) in yields of 75–100%. Pyrogallol trimethyl ether and 2-car-



(30)

(LIX)

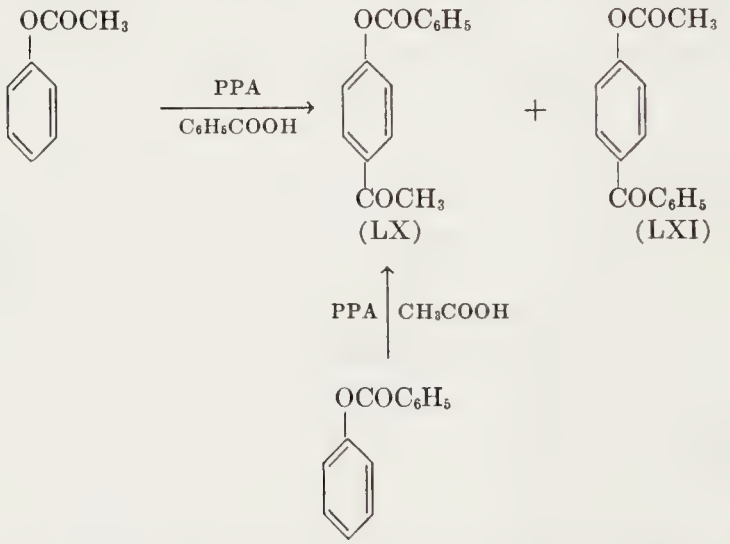
X = —H, —OCH_3; R = —$COCH_3$, —COC_6H_5, —$COCH_2CH_2COOCH_3$

bethoxycyclohexanone could not be induced to react (60). Anisole also has been acylated with crotonic, sorbic, butyric, and caproic acids, with yields of the *p*-hydroxyketones ranging from 60 to 90% (46).

Snyder and Elston (53,148) also investigated the general conditions for acylation with PPA. Reactive compounds like phenol and thiophene were found to give acyl derivatives in yields of about 70% at 75°. Benzene required such high temperatures that self-condensation of the aryl aliphatic ketones so produced ensued. Dev (46) reported the conversion of toluene to *p*-methylacetophenone in 75% yield by heating with acetic acid and PPA at 85° for $2^1/_4$ hr. In acylations with benzoic acid (148) self-condensation was not possible, and the reactions were successful at 160° (70% of *p*-methylbenzophenone). Electron-withdrawing substituents in the aromatic acid reduced its activity, as shown by the failure of *p*-nitrobenzoic acid to acylate phenol even at 160°. The double bond in cinnamic acid had a similar effect; acylation of anisole occurred, but not of benzene. *p*-Hydroxyacetophenone was obtained from phenol and acetic acid in 69% yield, but with caproic acid the yield of the ketone was only 35%, along with 18% of the ester. Benzoic acid and phenol gave 51% of the *p*-hydroxyketone and a trace of the ester when the reaction at 75° was run for 20 hr. *p*-Cresol and benzoic acid gave only the ester,

and none of the *o*-hydroxyketone could be found. *m*-Cresol and caproic acid gave both *o*- and *p*-hydroxyketones (22 and 6% yields, respectively). The ring positions of aromatic amides and amines, except diphenylamine, were inactive; diphenylamine gave about 15% of a dibenzoyldiphenylamine in which the benzoyl groups were believed to be in the *p,p'*-positions.

Gardner (61), in a study of the Fries rearrangement, concluded that the yields with PPA are no higher than with aluminum chloride, but that there is a greater tendency toward *para* rearrangement with PPA; *ortho* rearrangement was shown not to be an intermediate step in *para* rearrangement. Phenyl benzoate gave *p*-hydroxybenzophenone, along with its benzoate. With PPA at 90° for 1 hr., phenyl acetate and benzoic acid gave 21% of the benzoate of *p*-hydroxyacetophenone (LX) as the chief product, along with 3% of the acetate of *p*-hydroxybenzophenone (LXI), whereas phenyl benzoate and acetic acid gave only 21% of the benzoate of *p*-hydroxyaceto-



(31)

phenone (LX). These results show that the acetate undergoes rearrangement much more readily than the benzoate.

Nakazawa and his collaborators have studied a variety of acylations of phenols and Fries rearrangements of esters at 100° for periods of 10–20 min. Tables V and VI (114) indicate that with simple aliphatic esters the yields in both reactions are at the maximum with propionic acid. Acylations of phenol by a number of substituted benzoic acids also were studied (113), and the observations are

TABLE V
Reaction Products from Phenol and Carboxylic Acids and PPA
(yields in per cent)

Carboxylic acid	2-Acyl derivative	4-Acyl derivative	Phenyl ester
Acetic	20	65	3
Propionic	5	81	3
Butyric	5	76	15
Valeric	2	58	35
Caproic	—	40	57
β-Phenylpropionic	13	18	67
Phenylacetic	4	19	74
Benzoic	1	9	90

TABLE VI
Reaction Products from the Rearrangement of Phenyl Esters by PPA
(yields in per cent)

Phenyl ester	2-Acyl derivative	4-Acyl derivative
Acetate	20	53
Propionate	13	61
Butyrate	13	45
Valerate	6	40
Caproate	2	36
β-Phenylpropionate	1	10
Phenylacetate	1	6
Benzoate	1	6

shown in Table VII. It is to be noted that under the conditions employed, phenol and benzoic acid gave only a 16% yield of the *p*-hydroxyketone. As the table indicates, methoxyl and methyl groups in the *ortho* and *para* positions assisted the reaction but had little effect in the *meta* position. In the *para* position a hydroxyl group assisted, but not in the *ortho* or *meta* position. Halogen and nitro groups in any position interfere.

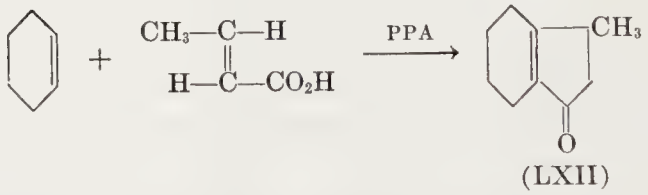
The acylation of α-naphthol and a number of polyhydroxy aromatic compounds, such as catechol, resorcinol, and pyrogallol and various of their derivatives, has been studied by several investigators

TABLE VII
Reaction Products from Phenol and Substituted Benzoic Acids and PPA
(20 min. at 100°C.; yields in per cent)

Substituent in benzoic acid	4-Acyl-derivative	Phenyl ester
o-NO_2	0.1	11
o-Cl	13	78
o-H	16	68
o-CH_3	47	38
o-OH	2	78
o-OCH_3	61	5
m-NO_2	1	11
m-Cl	5	42
m-H	16	68
m-CH_3	19	67
m-OH	7	41
m-OCH_3	15	65
p-NO_2	—	2
p-Cl	2	26
p-H	16	68
p-CH_3	24	59
p-OH	47	12
p-OCH_3	75	13

(87,112,116,118). Extremely good yields were obtained in many instances. An extensive tabulation of Friedel-Crafts acylations with various reagents including PPA has been published (120).

A few olefins have been acylated in PPA. Dev (47) obtained Δ^1-acetylcyclohexene and Δ^1-acetylcyclopentene in yields of 50–60 and 20–27%, respectively, from the acids and olefins. With unsaturated acids it was possible to effect a one-step synthesis of tetrahydroindanones from cyclohexene. The example shown (LXII)



(32)

(LXII)

was obtained in 55–60% from a reaction carried out at 57 ± 2° for $^1/_2$ hr. Similar compounds were made from other acids and from

cyclopentene. Benzoic acid and cyclohexene gave tetrahydrofluorenone, which could also be obtained from benzoylcyclohexane and PPA in 65% yield.

E. BECKMANN REARRANGEMENT

The Beckmann rearrangement in PPA was first effected by Werber (174), but difficulties in isolation of the water-soluble product (caprolactam) prevented recognition of the value of the reagent in this process until Horning and Stromberg (81) reexamined its use. Benzophenone oxime was found to rearrange quantitatively in PPA at 130° within 10 min., and many other ketoximes also gave excellent results. Even aldoximes were found to rearrange in PPA (82), making it possible for the first time to employ the rearrangement in establishing the configuration of aldoximes. Other reagents only dehydrate aldoximes, and previously the configuration of an aldoxime had to be established by the Hantsch method (71), in which the aldoxime acetate is treated with alkali to give the nitrile from the *syn*-oxime ester and either no reaction or regeneration of the oxime from the ester of the *anti*-oxime. The rearrangement of the oximes of benzaldehyde with PPA confirmed the previously assigned configurations. The liquid *syn*-oxime (LXIII) with PPA at 130° gave 9% of formanilide (LXIV) and 25% of benzamide (LXV), but the solid *anti*-oxime (LXVI) gave 40% of benzamide (LXV) and no

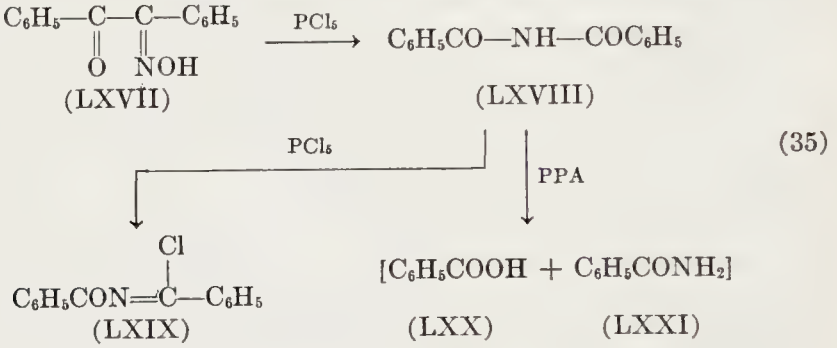
$$\underset{\text{(LXIII)}}{C_6H_5{-}C(=NOH){-}H} \xrightarrow[130°C.]{PPA} \underset{\text{(LXIV)}}{C_6H_5NHCHO} + \underset{\text{(LXV)}}{C_6H_5CONH_2} \qquad (33)$$

$$\underset{\text{(LXVI)}}{C_6H_5{-}C(=N{-}OH){-}H} \xrightarrow[130°C.]{PPA} 40\%\ \text{(LXV)} \qquad (34)$$

formanilide. It is evident that part of the *syn*-oxime isomerizes to the *anti*-oxime before rearrangement, whereas the stable *anti*-oxime rearranges without isomerization. The hydrochloride of the *anti*-oxime gave LXV in 80% yield in 5 min. at 130°.

A number of Beckmann transformations which are difficult or impossible with other reagents have been carried out successfully in PPA (83). Thus, the oxime of α-tetralone, which with other reagents yields α-naphthylamine, was rearranged to homocarbostyril in 91% yield (83). The oxime of 3,5-dimethyl-2-cyclohexenone, believed

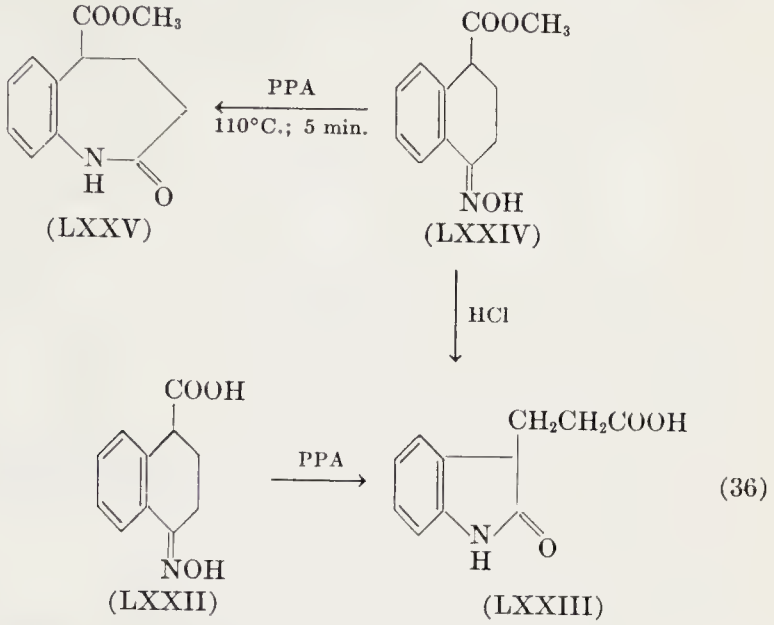
to be a mixture of *syn* and *anti* forms, gave only the unsaturated lactam corresponding to the *syn* form (83). Similarly, the *syn*-oxime of 2-cyclohexenone gave the expected unsaturated lactam, but the *anti*-oxime gave no identifiable products (51). The oxime of fluorenone, which requires unusually strenuous conditions with the ordinary reagents, rearranged to phenanthridone in 93% yield when heated with PPA at 180° (83). The use of nitromethane as a source of hydroxylamine in the formation of this oxime was mentioned earlier (Section III). The α-oxime of benzil (LXVII), which with phosphorus pentachloride gives the chloro derivative, *N*-benzoylbenzimido chloride (LXIX), with PPA gave benzoic acid and benz-



amide, which was considered to be the result of hydrolysis of the normal rearrangement product (LXVIII). However, this interpretation has been questioned (53) on the basis of the later discovery that PPA under approximately these conditions causes the hydrolysis of nitriles to amides in excellent yields (147). The fact that desoxybenzoin reacted with nitrous acid in PPA at room temperature (5 hr.) to give benzoic acid and benzonitrile suggested that the oxime (LVII) was cleaved by PPA to these products and the nitrile was hydrolyzed to benzamide under the more severe conditions (53). Phenylacetone oxime gave 29% of *N*-benzylacetamide, the normal rearrangement product, but not often obtained with other reagents.

PPA has been used in a study of the ring contraction which occurs in the Beckmann rearrangement of the oxime of 4-carboxy-1-tetralone (LXXII). With PPA, oxindole-3-propionic acid (LXXIII) was produced (108). However, the methyl ester (LXXIV) gave the expected seven-ring lactam (LXXV). Treatment of LXXV with concentrated hydrochloric acid converted it to LXXIII by exchange between the ester and amide groups and hydrolysis of the ester.

Evidently PPA causes the exchange of acid and amide groups in the transformation of LXXII to LXXIII.

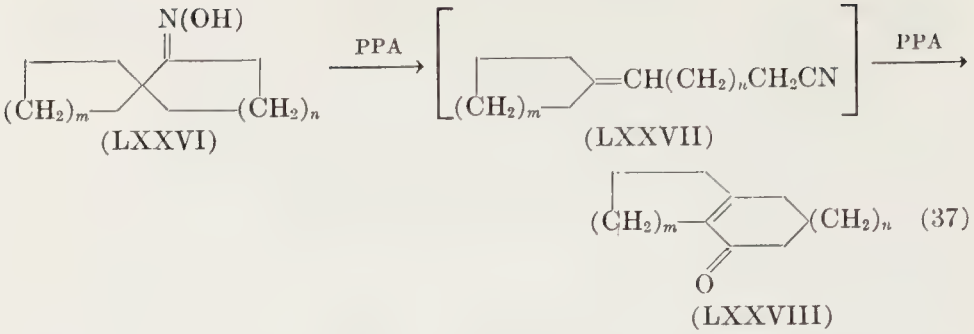


(36)

Oximes of heterocyclic ketones have been found to rearrange normally (18), except in some cases where the hetero atom is conjugated with the oxime function (166). However, lactamoximes, in which a nitrogen atom is directly connected to the oxime carbon atom, are rearranged to cyclic ureas in excellent yields (20).

Even anthraquinone dioxime has been rearranged in PPA (138). The product was the eight-membered dilactam, dianthranilide, showing that the hydroxyl groups in the dioxime are *trans* to each other. 1.5-Dichloroanthraquinone formed two dioximes which, on the basis of the rearrangement in PPA, were considered to be the *trans-trans* and *cis-trans* compounds.

A few oximes have given unexpected products in attempted PPA catalyzed Beckmann rearrangements. The mixed oximes of *o*-phenylbenzophenone, which gave the normal products, *o*-phenylbenzanilide and *o*-benzamidobiphenyl (ratio, 70:30) on rearrangement with phosphorus pentachloride, with PPA gave instead the cyclization products, fluorenone anil and 9-phenylphenanthridine (ratio, 80:20) (145). Oximes of certain spiroketones (LXXVI) underwent skeletal rearrangements, as shown in eq. 37 (78). Apparently the first step was cleavage of the oxime to the unsaturated nitrile



(LXXVII) which underwent cyclization and hydrolysis in the PPA yielding the bicyclenone (LXXVIII). Unsaturated nitriles of the structure LXXVII were shown to yield the ketones LXXVIII upon treatment with PPA. A cleavage similar to that postulated was observed with the oxime of 2,2-diphenylcycloheptanone, which gave the saturated amide 7,7-diphenylheptanamide along with unidentified products; presumably cleavage produced the saturated nitrile, which was hydrolyzed to the amide.

The hydrate of a 1,4-cyclohexanedione dioxime hydrochloride gave only 2-chloro-1,4-phenylenediamine upon reaction with PPA (109).

The use of unnecessarily severe conditions in the Beckmann reaction with PPA may cause further degradation of the amide (see Section IV-F).

F. MISCELLANEOUS APPLICATIONS

In view of the similarity in mechanism between the Beckmann and Lossen rearrangements, it is not surprising that PPA is capable of transforming hydroxamic acids to amines (150). The hydroxamic acid may be generated *in situ* by the reaction of hydroxylamine with the acid or a derivative of it such as an ester, amide, or acid chloride. Heating of a mixture of the acid, a hydroxylamine salt, and PPA to the temperature at which rapid evolution of carbon dioxide began, usually about 150–170°, for 5 min. usually sufficed. Aromatic acids, except the *o*- and *p*-nitro derivatives, usually gave good yields of the primary amines (149). The fact that amides gave the reaction suggested that the complete degradation of a diarylketone to two molecules of primary aromatic amine could be effected in a single step, and the degradation of benzophenone to aniline was carried out in 66% yield.

Although the only product isolated from salicylic acid and hydroxylamine in PPA was benzoxazolone (150), at 180–200° 3,5-dihydroxybenzoic acid gave 3,5-dihydroxyaniline, the hydrolysis of which provided a route from the acid to phloroglucinol (170).

The use of PPA instead of sulfuric acid as the medium for the Schmidt reaction of ketones has been recommended (34,53). Stirring of equimolecular amounts of benzophenone and sodium azide with PPA at 50° for $2^1/_2$ hr. gave 80% of pure benzanilide, with the recovery of almost an additional 20% in slightly impure state from the mother liquors (53). A number of other ketones have been converted to amides in yields running as high as 98% by reaction with sodium azide and PPA at an unspecified temperature, and an account of some abnormal Schmidt reactions has been promised (34). At 50°, benzoic acid reacted with PPA and sodium azide to give 48% of aniline and a considerable amount of diphenylurea; *p*-nitrobenzoic acid was recovered unchanged.

Amines are converted to amides by heating with carboxylic acids and PPA (149). The yields are highest with weakly basic amines; thus, in reactions conducted by raising the temperature from 25 to 160° over a period of 20 min. before hydrolysis with ice, benzoic acid and aniline gave no amide, but benzoic acid and 2,4-dinitroaniline gave 98% of the substituted anilide. Acetic acid gave 92% of the 2,4-dinitroanilide. *o*- and *p*-Nitrobenzoic acids failed to react; *m*-nitrobenzoic acid gave an 8% yield of the amide with *p*-nitroaniline. In reactions of *p*-nitroaniline, the ease of amide formation appeared to increase with the strength of the acids used. Benzoic acid did not give the amide with *N*-methyl-*o*-nitroaniline; ring acylation occurred instead (1).

Simple aliphatic and aromatic nitriles are converted to amides in yields ranging to 96% or better by heating for 1–2 hr. at 100° with PPA (147). Only sterically hindered nitriles, such as mesitonitrile and 2,4,6-trisopropylbenzonitrile, failed to react. Acetone cyanohydrin gave 31% of α-hydroxyisobutyramide when the reaction was run for 18 hr. at room temperature, but at 85° for $^1/_2$ hr. it gave 2,2,5,-5-tetramethyl-4-oxazolidone, presumably the result of condensation of the hydroxyamide with acetone liberated by reversal of the cyanohydrin. The hydrolysis of 4-nitro-2,5-dimethoxybenzonitrile to the amide could be accomplished in good yield only with PPA (170). β-Ketonitriles have been hydrolyzed to β-ketoamides (72,147), usually

in very good yields; it has been reported that an alternative hydrolysis with boron fluoride and aqueous acetic acid yields β-ketoamides of slightly higher purity than those obtained with PPA (72).

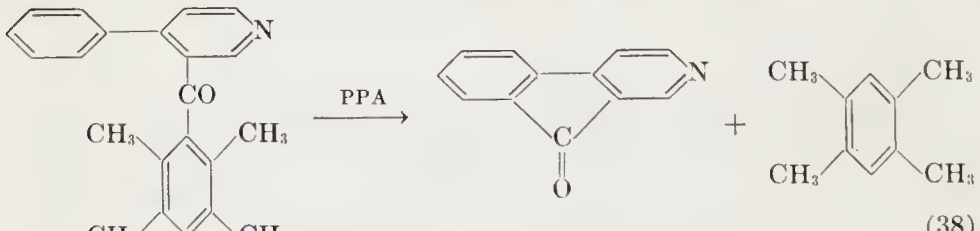
Among the most interesting esterifications effected with PPA are those of α-amino acids and benzyl alcohol, brought about by heating at 90–105° for 4 hr. (54). The hydrochlorides of the benzyl esters of *dl*-phenylalanine (65%), *l*-cysteine (45%), *l*-phenylalanine, *l*-leucine, and *l*-tyrosine have been prepared in this way. A number of monophosphate esters of amino alcohols, hydroxy esters, hydroxyamides, and hydroxynitriles have been prepared from PPA (31). The preparations of phosphate esters of alcohols (32) including glucose (142) were among the earliest uses of PPA.

Not many applications of the dehydration of alcohols to olefins with PPA have been reported. Among them are the conversion of decahydro-2-naphthol to $\Delta^{9,10}$-octalin (43), of 1,2-benzocyclooct-1-en-3-ol to 1,2-benzocycloocta-1,3-diene (90), and of a hydroxyketone to an unsaturated ketone (172).

PPA has been used instead of acetic hydride in the nitration of malonic esters (97), and instead of phosphorus halides in the bromination of acids (144).

Dypnone has been prepared from acetophenone and PPA at 70–80° (14).

Hindered ketones, such as mesityl ketones, are hydrolyzed to acids and hydrocarbons by PPA (123). When the reaction was applied to 3-duroyl-4-phenylpyridine (LXXIX), removal of the duryl group was followed by cyclization to 2-azafluorenone (LXXX) (58).



(LXXIX) (LXXX) (38)

V. Experimental

A. SELECTION OF EXPERIMENTAL CONDITIONS

Inasmuch as PPA has been used for such a variety of reactions, it is difficult to generalize on the choice of experimental conditions.

However, one of the advantages of PPA is that it is mild enough that variations in temperature and time of reaction are less critical than with many other reagents. Many of the reactions are carried out at steam bath temperatures, the times being adjusted as necessary according to the reactivity of the organic reagents. In any particular instance, it may be found that a lower or a higher temperature is beneficial.

Koo (102) has compared the conditions most favorable to the formation of various indenes, 1-indanones, 1-tetralones, benzosuber-5-ones, and anthraquinones in PPA cyclizations. He concluded that the reactivities of both the aryl nucleus and the carbonyl group are to be considered in determining the severity of the conditions required. When the carbonyl group was an unusually reactive one, such as that of the formyl group in an α-formyl ester or that of a malonic ester group, the cyclization on to an unactivated phenyl group could be effected by reaction in PPA at 0–10° for periods between 5 and 30 min. When the carbonyl group was that of a methyl ketone or an acetoacetic ester, a period of about 30 min. at room temperature sufficed. With phenyl ketones and carboxylic acids, heating to 60–70° for $^1/_2$–1 hr. usually was required. The conversion of benzoylbenzoic acids to anthraquinones required several hours at 80–90°; presumably the deactivating effect of the carbonyl group on the aromatic nucleus is the important factor in increasing the stability of these acids. He also noted that liquids or low melting solids usually underwent cyclization under milder conditions than their higher melting analogs.

Several authors have noted the formation of intense colors during PPA cyclizations, and Koo (102) and Uhlig (168) have suggested the use of the color development as a means of determining the severity of the conditions to be employed. A trace of the substance is added to 10–30 parts of polyphosphoric acid at room temperature in a test tube and the color is observed. If a dark color develops immediately, it is probable that the cyclization can be carried out in the cold. If the color develops slowly, cyclization at room temperature may be considered. If little or no color develops in the test at room temperature, the reaction may require heating. In a preparative run, the color development may be used to determine the time of reaction. At the optimum point the color of the mixture has reached its maximum intensity, usually a deep red but sometimes a bright yellow or

even a deep purple. Beyond this point the mixture may begin to assume a "dirty" appearance as a brown or black material begins to form, and the reaction may be terminated just as this change begins.

Unfortunately, in many of the other reactions in which PPA has been used no such ready indications of the progress of the reaction are available. In testing the utility of the reagent in a new reaction, it is probably best to conduct several small-scale trials under conditions of increasing severity and judge the course of the process by whatever means may be appropriate (spectral analysis, vapor phase chromatography, isolation of the product, etc.).

As indicated above, solvents have not been much used with PPA, an excess of the latter serving this purpose. Although the relative amounts of PPA and the organic reagent have varied over a considerable range, a ratio of 10:1 may be taken as a point of departure. Most organic compounds seem reasonably soluble in PPA, and only moderate stirring is required to bring them into reaction. With a very high melting material, it may be desirable to grind the reagent to a fine powder and to stir vigorously during and after the addition to the PPA.

The isolation of the product usually is effected by adding the reaction mixture to a weight of water or ice and water somewhat greater than the weight of PPA employed. The product is separated by filtration, extraction, or continuous extraction, as the properties of the product may dictate. In the isolation of bases, it may be necessary to dilute the mixture further and neutralize the acid before the product can be removed. However, if the phosphate salt of the base happens to have a low solubility, as in the case of benzoflavine (Section III), its isolation is very simple. Precipitating agents, when they are known, might be used in other instances to avoid the neutralization step.

Although PPA can be made in various ways, as indicated in Section I, the senior author has preferred to use the commercial product, obtained from the Victor Chemical Works, Chicago, Ill. This substance is available as a transparent, colorless or nearly colorless liquid of high viscosity. Earlier lots were labeled as having a phosphorus pentoxide equivalent in the range of 82–84%; more recent samples are labeled as equivalent to 115% of orthophosphoric acid, which corresponds closely to an 84% phosphorus pentoxide equivalent. The viscosity of the liquid displays a curious temperature dependence.

After being cool for some time, it may become too viscous for easy pouring, but if the container is warmed on a steam bath the liquid soon becomes conveniently mobile and will remain so at room temperature for days. Bottles of the reagent which have been opened repeatedly to moist air develop a white crust at the top. Although this material has not been characterized, so far as the authors know, it probably is a mixture of hydrolysis products. Ordinarily the liquid can be poured from beneath it, and its presence seems to do no great harm.

If the reagent must be prepared in the laboratory, the most convenient method appears to be heating a mixture of phosphorus pentoxide and 85% phosphoric acid in a flask protected from atmospheric moisture. Gilmore and Horton (67) added the acid, apparently portion-wise, to the anhydride with occasional moderate cooling in water and then heated the mixture, with occasional stirring, on a steam bath for 2–4 hr. After 2 hr. some solid material remained undissolved, but its presence did not interfere with the use of the reagent in cyclizing aryl-substituted acids to ketones. However, in some uses, such as the conversion of nitriles to amides (Section IV-F), the solid material, if it is phosphorus pentoxide, might have a deleterious effect. The use of 1.5 g. of phosphoric anhydride per milliliter of 85% orthophosphoric acid yields a PPA mixture of approximately the anhydride content of the commercial PPA.

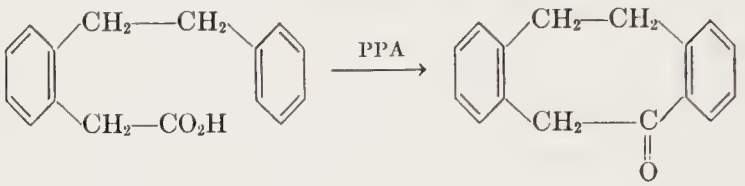
In a cyclization of 3-β-naphthylcyclopentan-1-one-2-acetic acid, reported in 1938, Koebner and Robinson (100) dissolved the acid (6 g.) in syrupy phosphoric acid (20 ml.) by gentle heating and added "gradually but quickly," and with vigorous shaking, phosphoric oxide (50 g.). The temperature rose above 100°; after an hour the mixture was treated with ice and the product was isolated. It has not been established whether the cyclization in this instance was brought about by the interaction of the P_4O_{10} with the carboxylic acid or whether PPA was the active reagent. It is possible that the procedure represents a more drastic one than the use of pre-formed PPA and that it should be considered when the latter reagent proves ineffective.

B. EXPERIMENTAL PROCEDURES

Cyclization of Aryl-Substituted Acids. The preparation of α-tetralone in 75–86% yield from γ-phenylbutyric acid and polyphosphoric

acid at temperatures near 90° is described in *Organic Syntheses* (155). Koo (102) has reported that by operation at 70° and increasing the reaction time from about 7 min. to 30 min. the yield is raised to 93%. Cyclization to five- and seven-membered ring ketones ordinarily is effected by the use of conditions similar to those employed in the procedures just mentioned. The following appears to be the only example of a PPA cyclization producing an eight-membered ring ketone in good yield. It employs a much larger ratio of PPA to acid (50:1) than is needed with the smaller-ring compounds.

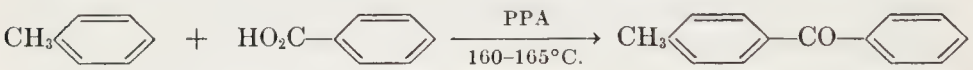
1,2,5,6-Dibenzo-1,5-cyclooctadiene-3-one (35):



(39)

Five g. of *o*-(β-phenylethyl)phenylacetic acid (35) was dissolved in 250 g. of hot PPA, and the yellow solution was stirred on a steam bath for 2 hr. Addition of the cooled solution to 600 ml. of ice water caused the separation of a white solid, which was collected and washed with 10% sodium carbonate solution and water. After drying, the product was crystallized from aqueous methanol to give 4.3 g. (93%) of the ketone in the form of colorless needles melting at 92–95°. The analytical sample, prepared by two recrystallizations from methanol and sublimation at 130° (0.5 mm.), melted at 95.2–96.5°.

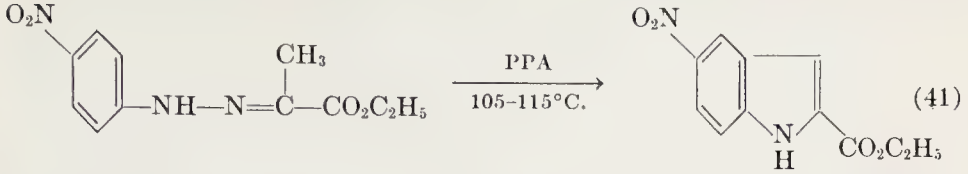
Intermolecular Acylation. 4-Methylbenzophenone (53):



(40)

A mixture of 10 g. (0.109 mole) of toluene, 23 g. (0.188 mole) of benzoic acid, and 75 g. of PPA was stirred vigorously at 160–165° for 3 hr. in a flask equipped with a mechanical stirrer and a reflux condenser with a drying tube. The mixture was allowed to cool and was treated with an excess of cold water, and the oil which separated was washed with water, dilute sodium hydroxide, and water. After distillation at 114–116° (0.4 mm.), it solidified to 15.0 g. of *p*-methylbenzophenone, m.p. 59°.

Fischer Indole Synthesis. Ethyl 5-nitroindole-2-carboxylate (126):



(41)

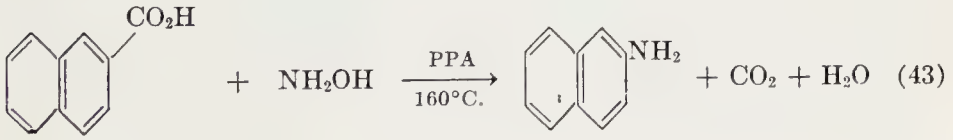
A mixture of 35 g. of finely powdered ethyl pyruvate *p*-nitrophenylhydrazone and 300 g. of PPA was warmed in an oil bath in a flask equipped with a powerful stirrer. As the temperature reached 90°, an exothermic reaction started and the mixture became semisolid. By means of an ice bath, kept at hand for the purpose, the temperature of the mixture was kept below 125°. After being stirred for $^1/_2$ hr. at 110–125°, the mixture was diluted with ice water. The solid was collected, washed thoroughly with water, and dried. To remove any unchanged phenylhydrazone, the solid was digested with 150 ml. of boiling 95% ethanol, and the extract was discarded. The residue was dissolved in 1 liter of boiling 95% ethanol under reflux; the rate of solution was quite low. The solution was filtered hot and concentrated to a volume of 500 ml. The concentrate was cooled and stored in a refrigerator until crystallization was complete. The indole derivative was obtained as yellow crystals, m.p. 220–223°, weighing 18.7 g. (57%). The analytical sample melted at 225–226°.

Beckmann Rearrangement. Benzanilide (81):

(42)

A mixture of 2 g. of benzophenone oxime and 60 g. of PPA was heated with manual stirring at 130° for 10 min., and then poured into 300 ml. of water. The aqueous solution was extracted with 1:1 ether–ethyl acetate, and the organic layer was washed with water and saturated aqueous sodium chloride; after being dried over magnesium sulfate the solution was concentrated to dryness. Essentially pure benzanilide, m.p. 161–162°, was obtained in quantitative yield as a colorless crystalline solid.

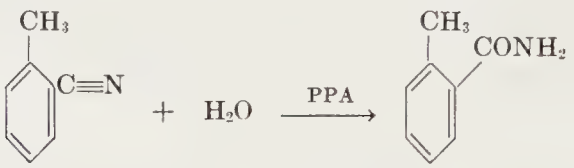
Lossen Rearrangement. β-Naphthylamine (150):



(43)

To a mixture of 1.74 g. of hydroxylamine hydrochloride and 4.0 g. of β-naphthoic acid was added 50 g. of PPA. The mixture was stirred mechanically and the temperature was slowly raised. Near 150° rapid gas evolution began, and the mixture turned brown. At 160° gas evolution had ceased, and the mixture was poured over 250 g. of crushed ice. The resulting solution was filtered from a small amount of brown solid and neutralized with potassium hydroxide. The precipitated β-naphthylamine weighed 2.7 g. (81%) and melted at 107–109°.

Hydrolysis of a Nitrile to an Amide. *o*-Toluamide (147):



(44)

A mixture of 5.0 g. of *o*-tolunitrile and 6.5 g. of PPA was heated with stirring at 115° for $1^1/_2$ hr. The colorless solution was diluted with ice water to a volume of 125 ml. The solid which separated was collected and combined with a second fraction which separated when the aqueous solution was neutralized with potassium hydroxide. After being washed with water, dilute sodium carbonate, and finally again with water, the dried solid weighed 5.5 g. (95%) and melted at 137–140°. One recrystallization from ethanol raised the m.p. to 140°.

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THE WITTIG REACTION*

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I. Introduction**

Wittig and Schöllkopf (41) showed that alkylidene- and arylidenetriphenylphosphoranes (II), prepared by the action of a suitable base on an alkyl- or aralkyltriphenylphosphonium halide (I), react rapidly

$$\underset{\text{(I)}}{(C_6H_5)_3\overset{\oplus}{P}CHR_1R_2\ \overset{\ominus}{X}} \xrightarrow{\text{base}} \underset{\text{(II)}}{(C_6H_5)_3P{:}CR_1R_2} \xrightarrow{R_3R_4CO}$$

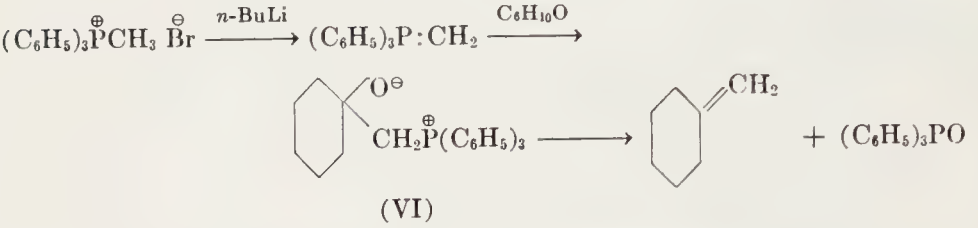
$$R_3R_4C(\overset{\ominus}{O})-R_1R_2C-\overset{\oplus}{P}(C_6H_5)_3 \longrightarrow \underset{\text{(IV)}}{(C_6H_5)_3PO} + \underset{\text{(V)}}{R_1R_2C{:}CR_3R_4}$$

(III)

* For recent review see (43).

** Phosphorus nomenclature used in this article: $R_4\overset{\oplus}{P}\ \overset{\ominus}{X}$ = phosphonium halide, e.g., $Me(C_6H_5)_3\overset{\oplus}{P}\overset{\ominus}{Br}$, methyltriphenylphosphonium bromide; R_3PO = phosphine oxide, e.g., $(C_6H_5)_3PO$, triphenylphosphine oxide; R_5P = phosphorane, e.g., $(C_6H_5)_5P$, pentaphenylphosphorane; $(C_6H_5)_3P{:}CH_2$, methylenetriphenylphosphorane.

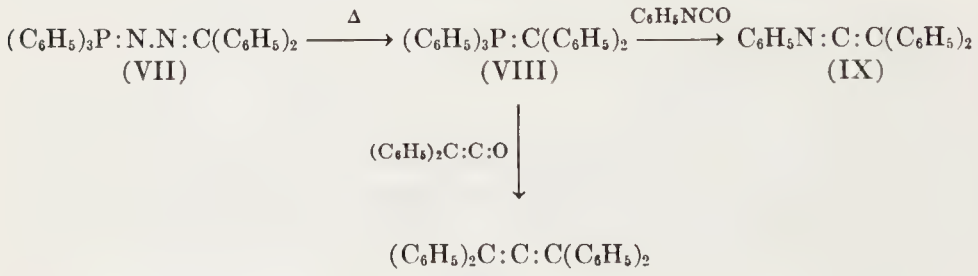
with aldehydes and ketones to give zwitterionic compounds (III) which eliminate triphenylphosphine oxide (IV) under mild conditions to form olefins (V). Thus, methyltriphenylphosphonium bromide with *n*-butyllithium in ether gave a solution of methylenetriphenylphosphorane, which, with cyclohexanone, gave the betaine (VI). This, on heating in suspension in ether to 65°, eliminated triphenylphosphine oxide and gave methylenecyclohexane in 48% yield.



(VI)

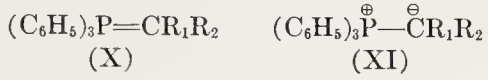
In marked contrast with other olefin syntheses, the position of the resulting double bond is not in doubt, even when it occupies an energetically unfavorable position. The above sequence of reactions proceeds under mild conditions and is singularly free from complication. It has become known as the Wittig reaction.

In 1919, Staudinger and Meyer (35) reported that the adduct (VII) of triphenylphosphine and diphenyldiazomethane on heating lost nitrogen to give diphenylmethylenetriphenylphosphorane (VIII), which was stable to ordinary carbonyl compounds but with phenyl isocyanate gave the keteneimide (IX) with the elimination of triphenylphosphine oxide. Lüscher (29) later heated the phosphorane (VIII) with diphenylketene at 140° in benzene under pressure to give an almost quantitative yield of tetraphenylallene. Although these may be regarded formally as the first reactions of their type to appear in the literature (29,30), the general olefin synthesis was developed by Wittig and is justly named after him.



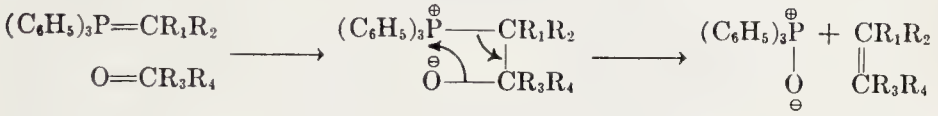
II. Mechanism

An alkylidenetriphenylphosphorane may be represented as a resonance hybrid of contributing structures such as X and XI. Reaction



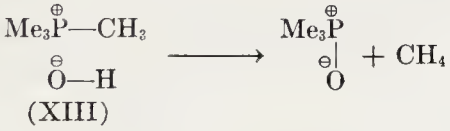
(X) (XI)

with a carbonyl compound involves attack by the nucleophilic carbon of the phosphorane on the carbonyl group and elimination of triphenylphosphine oxide then takes place, probably via a four-membered cyclic transition state.

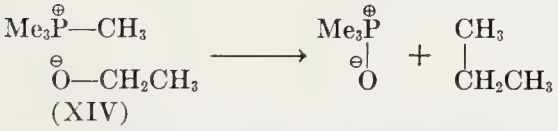


(XII)

Wittig and Haag (40) point out the analogy between this elimination and the thermal decomposition of quaternary phosphonium hydroxides (XIII) and alcoholates (XIV).

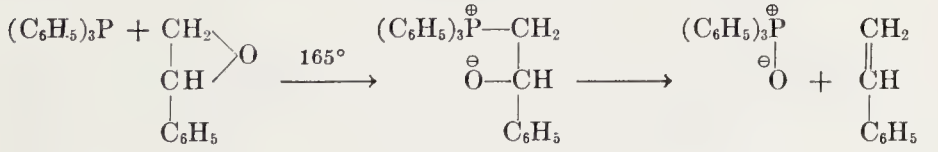


(XIII)



(XIV)

Zwitterions of type XII are probable intermediates in the action of triphenylphosphine on epoxides to give olefins (40). Triphenylphosphine and styrene oxide on heating together at 165° gave triphenylphosphine oxide (86%) and styrene (50%). Similarly, triphenylphosphine and β-phenylglycidic ester at 170° in the presence of hydroquinone gave a mixture of *cis*- and *trans*-cinnamic esters.

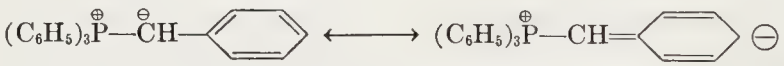


Alkylidenetrialkylphosphoranes, e.g., $(CH_3)_3P:CH_2$, react with carbonyl compounds to give zwitterionic compounds analogous to XII; but these will not eliminate trialkylphosphine oxides on heating. Wittig (41) ascribes this to hyperconjugation of the alkyl groups with the positively charged phosphorus atom reducing the electron affinity of that atom.

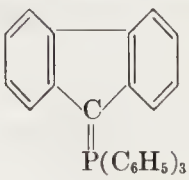
III. Scope and Limitations

A. THE TRIPHENYLPHOSPHORANE

Wittig reagents of the general formula $(C_6H_5)_3P:CR_1R_2$ are prepared by the action of a suitable base on the corresponding phosphonium halide. They are yellow to red crystalline compounds. The alkylidenetriphenylphosphoranes (R_1, R_2 = H or alkyl) are highly reactive; they rapidly absorb oxygen and, besides adding to aldehydes and ketones, react with water, halogen acids, alcohols, and other carbonyl compounds, e.g., esters. The arylmethylenetriphenylphosphoranes (R_1 = H; R_2 = aryl) are somewhat less reactive, e.g., benzylidenetriphenylphosphorane does not react with Michler's ketone (40), and the diarylmethylenetriphenylphosphoranes (R_1, R_2 = aryl) are stable to oxygen and ordinary carbonyl compounds but are decolorized by water, alcohols, and acids (35). This increasing stability is associated with increasing delocalization of the negative charge on the methylene carbon atom, e.g.



In extreme cases, e.g., fluorenylidenetriphenylphosphorane (XV) (18) and cyclopentadienylidenetriphenylphosphorane (XVI) (33), the phosphoranes may be prepared in aqueous solution and are remark-



(XV)

(XVI)

ably stable compounds. They are in general of no value in the Wittig reaction.

The wide reactivity of Wittig reagents has obvious bearing on their preparation and use in synthesis. The rapid uptake of oxygen has not been further investigated, but may be analogous to the action of sulfur on diphenylmethylenetriphenylphosphorane to give triphenylphosphine sulfide and thiobenzophenone (35).

$$(C_6H_5)_3P{:}C(C_6H_5)_2 \xrightarrow{S} (C_6H_5)_3PS + (C_6H_5)_2CS$$

With halogen acids the corresponding phosphonium halide is formed, whereas treatment with water leads to the elimination of benzene and the formation of an alkyldiphenylphosphine oxide.

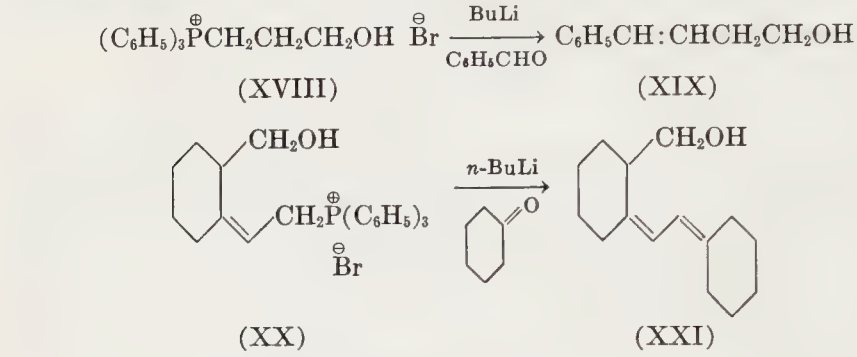
$$(C_6H_5)_3P{:}CH_2 \xrightarrow{HCl} (C_6H_5)_3\overset{\oplus}{P}CH_3 \ \overset{\ominus}{Cl}$$

$$\xrightarrow{H_2O} C_6H_6 + (C_6H_5)_2MePO$$

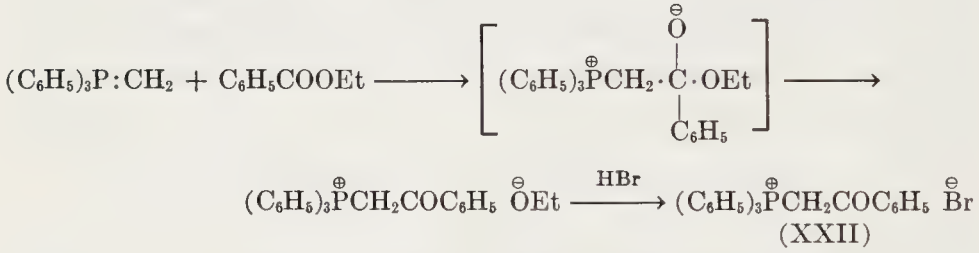
Alkyl halides add to alkylidenetriphenylphosphoranes to give phosphonium halides. Thus, butyllithium on the quaternary phosphonium bromide (XVII) is thought to give cyclobutyltriphenylphosphonium bromide (31). The addition is particularly rapid with allylic halides, but in most cases the product is a complex mixture of phosphonium halides probably arising via an exchange reaction between initially formed phosphonium halide and unreacted phosphorane (37).

$$\underset{\text{(XVII)}}{(C_6H_5)_3\overset{\oplus}{P}CH_2CH_2CH_2CH_2Br \ \overset{\ominus}{Br}} \xrightarrow{BuLi} (C_6H_5)_3\overset{\oplus}{P}\overset{\ulcorner\!\!\!

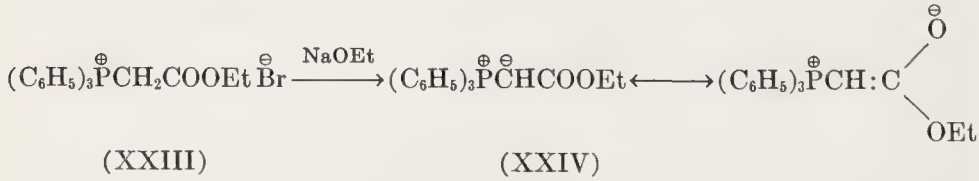
aldehyde gave the olefin (XIX) in 65% yield (37), whereas the hydroxyphosphonium bromide (XX) with butyllithium (1.9 mole) gave an 80% yield of the diene (XXI) on reaction with cyclohexanone (15). Conversion to the tetrahydropyranyl ether and formation of an acetal have been used to protect hydroxyl groups in the Wittig reaction (20).



Alkylidenetriphenylphosphoranes add rapidly to esters (41), and treatment of the intermediates with halogen acids gives β-ketoalkyltriphenylphosphonium halides, e.g., XXII. This reaction precludes the use of Wittig reagents containing an ester group in the same mole-



cule, except in the special case of the reagents derived from α-bromoesters, e.g., XXIV, where resonance of the type depicted stabilizes the phosphorane and deactivates the ester group. Thus, the phosphonium bromide (XXIII) with sodium ethoxide in ethanol gave with

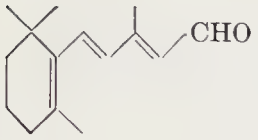


benzaldehyde a 77% yield of cinnamic acid (41). The vinylogous phosphonium bromide (XXV) from γ-bromocrotonic ester reacts sim-

ilarly (4), and the analogous phosphonium bromide (XXVI) has found use in a synthesis of vitamin A (38,42).

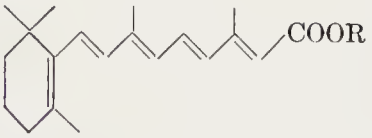
$$(C_6H_5)_3\overset{\oplus}{P}CH_2CH{:}CHCOOEt\ \overset{\ominus}{Br} \xrightarrow[C_6H_5CHO]{NaOEt} C_6H_5CH{:}CH{\cdot}CH{:}CHCOOEt$$

(XXV)



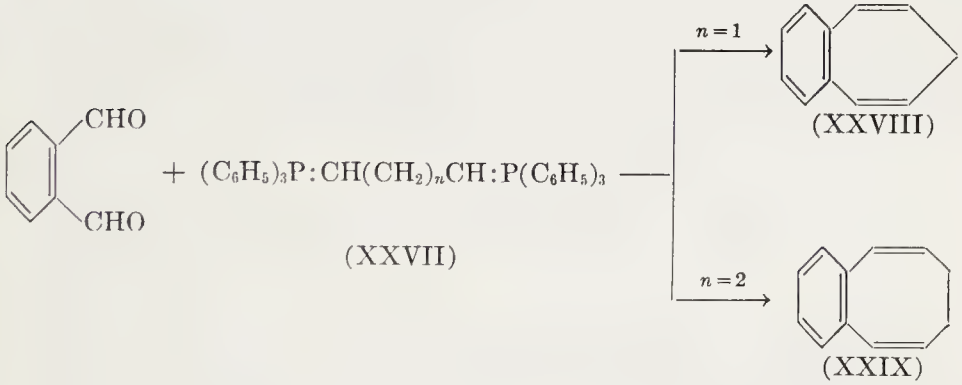
$$+ (C_6H_5)_3\overset{\oplus}{P}CH_2CMe{:}CHCOOR\ \overset{\ominus}{Br} \xrightarrow{NaOR}$$

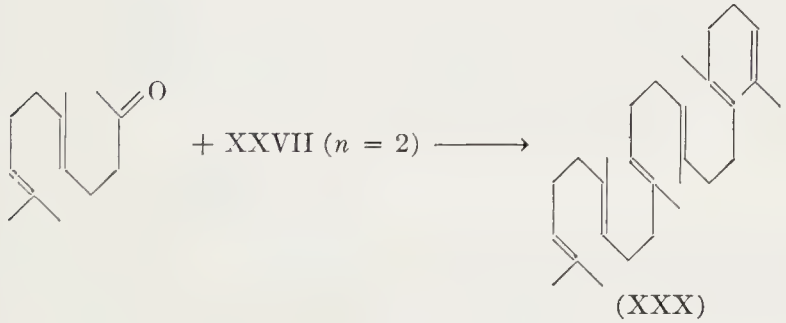
(XXVI)



$$\xrightarrow{LiAlH_4} \text{Vitamin A}$$

Bifunctional Wittig reagents have proved useful in several fields. The phosphoranes (XXVII, $n = 1, 2$) with *o*-phthalaldehyde gave the bicyclic hydrocarbons (XXVIII and XXIX) (38), whereas squalene (XXX) has been synthesized from geranylacetone and the phosphorane (XXVII, $n = 2$) (14,31,36).



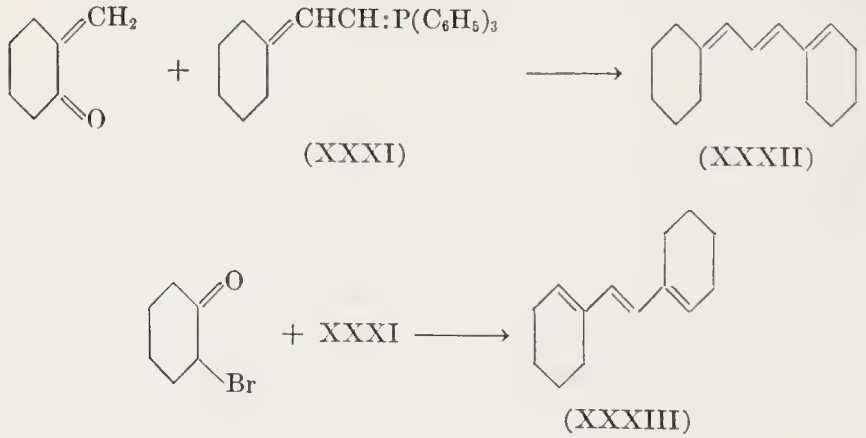


Olefins and acetylenes are unaffected by Wittig reagents, and many highly unsaturated alkylidenetriphenylphosphoranes have been used in this synthesis (see Section IV).

B. THE ALDEHYDE OR KETONE

The Wittig reaction proceeds without complication with saturated and unsaturated aliphatic and alicyclic aldehydes and ketones, with alkylaryl and diaryl ketones, and with aromatic aldehydes. Heterocyclic compounds have not yet been investigated. The aldehyde or ketone may also contain the following groups: hydroxy (16,37), acetoxy (23), methoxy, acetal (20), tetrahydropyranyloxy (20), dimethylamino (41), terminal acetylenic (11), and nuclear aromatic halogen and nitro (41). There may also be an ester group provided reverse addition is employed (6), the Wittig reagent being added to an excess of the carbonyl compound (see Section IV-B).

Few anomalous reactions are on record. The alkylidenetriphenylphosphorane (XXXI) gave with 2-methylenecyclohexanone the triene (XXXII) (21), and treatment of 2,4,5-triacetoxybenzaldehyde with the phosphonium bromide derived from γ-bromocrotonic ester in the presence of sodium ethoxide gave only 2-hydroxy-4,5-diacetoxybenzaldehyde (4). The former reaction is thought to be the only example yet encountered of 1,4-addition of a Wittig reagent to an α,β-unsaturated ketone. α-Halogenoketones may react with the elimina-



tion of hydrogen halide; 2-bromocyclohexanone with the phosphorane (XXXI) gave the triene (XXXIII) (37).

C. STEREOCHEMISTRY OF THE RESULTING OLEFIN

The formation of a mixture of *cis* and *trans* isomers in the Wittig reaction was first noted by Wittig (41) in the reaction of allylidenetriphenylphosphorane with benzaldehyde to give a 1:1 mixture of *cis*- and *trans*-1-phenylbutadiene, and has since proved to be a general phenomenon. The ratio of *cis* to *trans* isomer isolated may vary with the direction of coupling, the base used, and the temperature of reaction. Thus, acraldehyde with the phosphorane (XXXIV) gave only

$$\underset{\text{(XXXIV)}}{MeCH{:}CH(C{\equiv}C)_2CH{:}CHCH{:}P(C_6H_5)_3} + CHOCH{:}CH_2 \longrightarrow MeCH{:}CH(C{\equiv}C)_2CH{:}CHCH\overset{t}{:}CHCH{:}CH_2$$

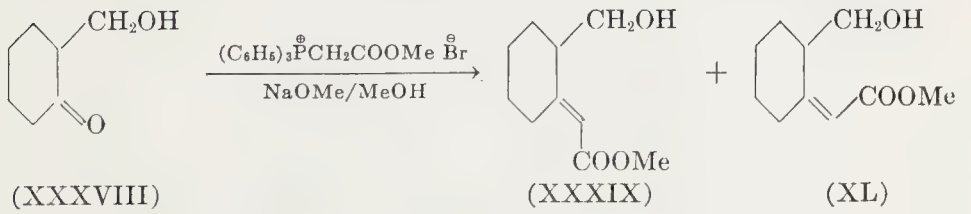
(XXXV)

$$\underset{\text{(XXXVI)}}{MeCH{:}CH(C{\equiv}C)_2CH{:}CHCHO} + (C_6H_5)_3P{:}CHCH{:}CH_2 \longrightarrow \underset{c\,\&\,t}{\text{XXXV}}$$

the *trans*-hydrocarbon (XXXV), whereas a mixture of *cis*- and *trans*-hydrocarbons resulted when allylidenetriphenylphosphorane reacted with the aldehyde (XXXVI) (8). Benzyltriphenylphosphonium bromide with butyllithium and benzaldehyde gave 30% of *cis*- and 70% of *trans*-stilbenes (40), but, with sodium ethoxide as the base in ethanol, 53% of *cis*- and 47% of *trans*-stilbenes were obtained. Temperature of reaction influenced the ratio of isomers isolated in the reaction of benzaldehyde with the biphosphorane (XXXVII): at room temperature a mixture of the three *cis-trans* isomers was obtained, but heating under reflux in ether for 1 hr. gave only the *trans-trans* isomer (31).

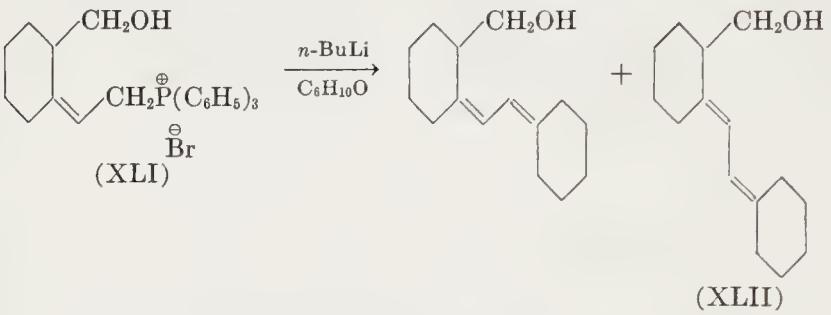
$$\underset{\text{(XXXVII)}}{(C_6H_5)_3P{:}CHCH_2CH_2CH{:}P(C_6H_5)_3} \xrightarrow{C_6H_5CHO} C_6H_5CH{:}CHCH_2CH_2CH{:}CHC_6H_5$$

In many Wittig reactions, only one of the possible isomers is formed, probably for steric reasons, e.g., 2-hydroxymethylcyclohexanone (XXXVIII) with carboxymethyltriphenylphosphonium bromide and sodium methoxide in methanol gave a product containing only 0.5% of the *cis* isomer (XL), detected by its ready lactone formation (15).



(XXXVIII) (XXXIX) (XL)

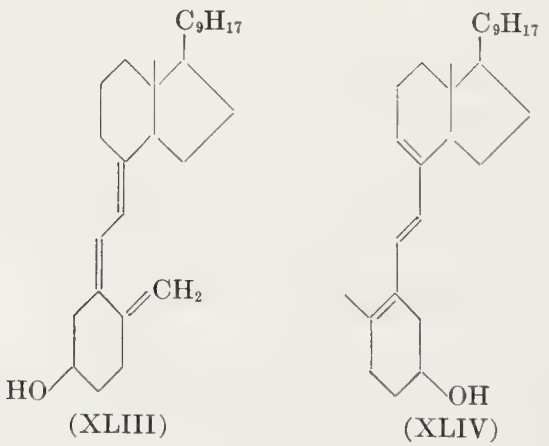
The formation of a phosphorane from an allyltriphenylphosphonium bromide may lead to *cis-trans* isomerization in the allyl group. The *cis*-allyltriphenylphosphonium bromide (XLI) with butyllithium (1.9 mole) and cyclohexanone gave, at 40°, a diene containing 25% of the *trans* isomer (XLII). The proportion of *trans* isomer was reduced to 5% by preparation of the phosphorane at −25° and warming to 0° after addition of the ketone (15).



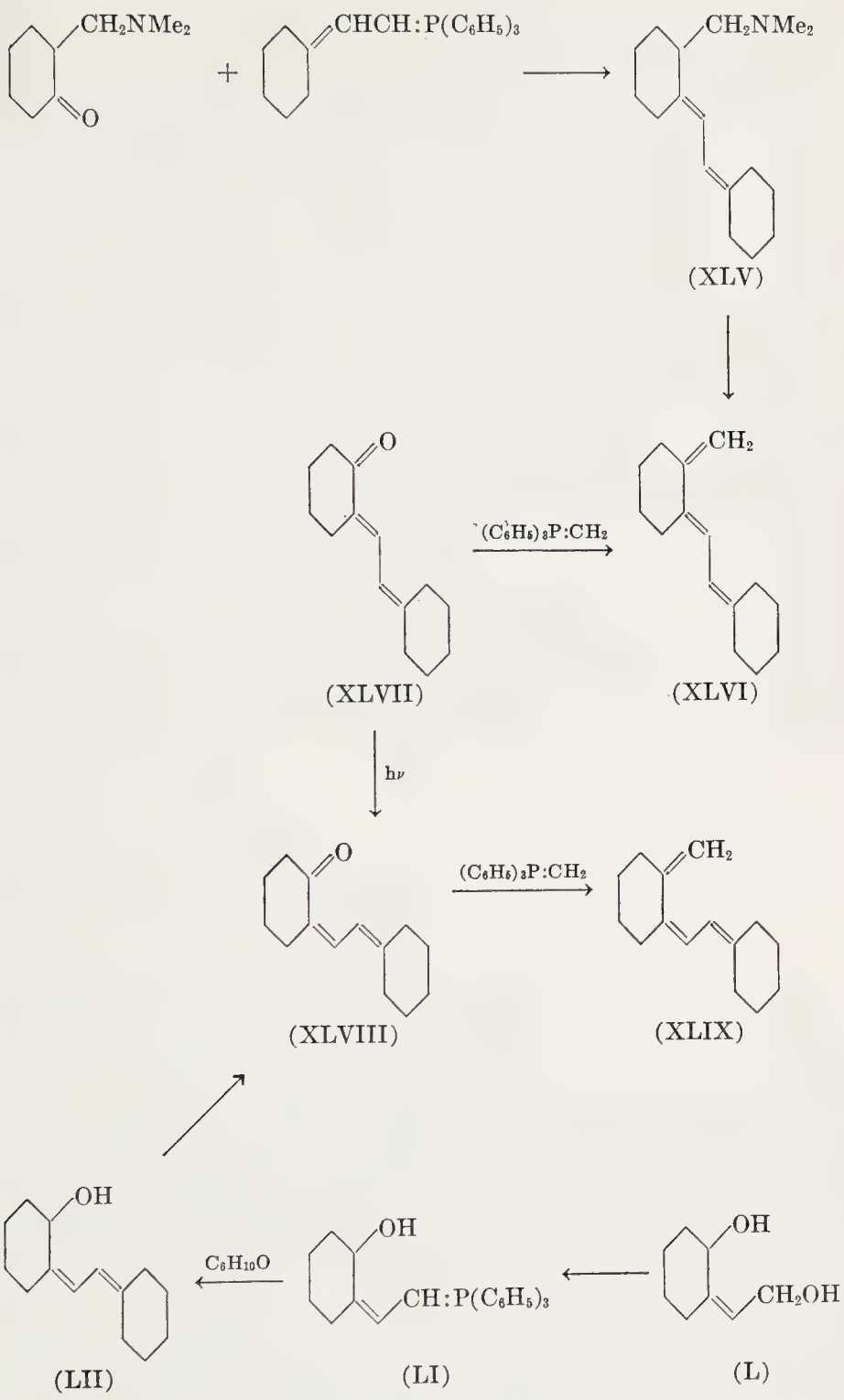
(XLI) (XLII)

IV. Some Applications of the Wittig Reaction

Methylenetriphenylphosphorane has been extensively used in the synthesis of compounds having exocyclic methylene groups. Cyclohexanone gave methylenecyclohexane in 48% yield (41), and in the steroid field exocyclic methylenes have been introduced at positions 3 (1,34), 7 (19,34), 12 (34), and 17 (34), and methylenes at positions 20 (34), 24 (2,19), and 25 (19). However, the most extensive applications of the Wittig reaction have been in the vitamin D field and in the synthesis of naturally occurring polyenes and polyenes for use in light absorption studies.



(XLIII) (XLIV)

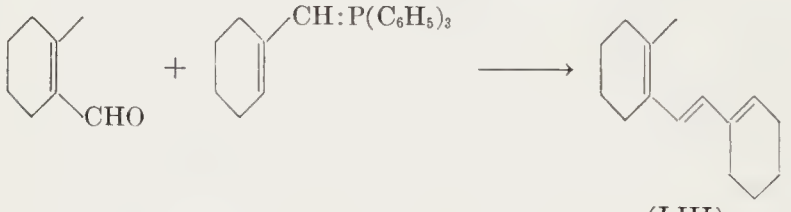

CH2NMe2
O
+
CHCH:P(C6H5)3
CH2NMe2
(XLV)
O
(C6H5)3P:CH2
CH2
(XLVII)
(XLVI)
hν
O
(C6H5)3P:CH2
CH2
(XLVIII)
(XLIX)
OH
C6H10O
OH
CH:P(C6H5)3
OH
CH2OH
(LII)
(LI)
(L)

A. THE VITAMIN D FIELD

Several model trienes have been synthesized for comparison of their spectra with those of the vitamins D (XLIII, λ_{max} 265 mμ) and of other irradiation products of ergosterol, e.g., tachysterol (XLIV, λ_{max} 280.5 mμ).

Cyclohexylidene-ethylidenetriphenylphosphorane with 2-dimethylaminocyclohexanone gave the diene–amine (XLV) which, on thermal decomposition of the corresponding quaternary ammonium hydroxide, gave the *trans*-triene (XLVI) having λ_{max} 270 mμ (17). The *trans*-triene was also obtained from the *trans*-dienone (XLVII) and methylenetriphenylphosphorane (17). The corresponding *cis*-triene (XLIX, λ_{max} 262 mμ) has been synthesized via the *cis*-dienone (XLVIII), obtained either from the *trans*-dienone by irradiation or by a nonphotochemical route involving conversion of the *cis*-diol (L) to the phosphorane (LI). This compound (LI), with cyclohexanone, gave the *cis*-dienol (LII), oxidation of which gave the *cis*-dienone (15,16).

Among other model trienes synthesized in this field is the tachysterol model (LIII), obtained from 1-cyclohexenylmethylenetriphenylphosphorane and 2-methylcyclohex-1-ene aldehyde (22).

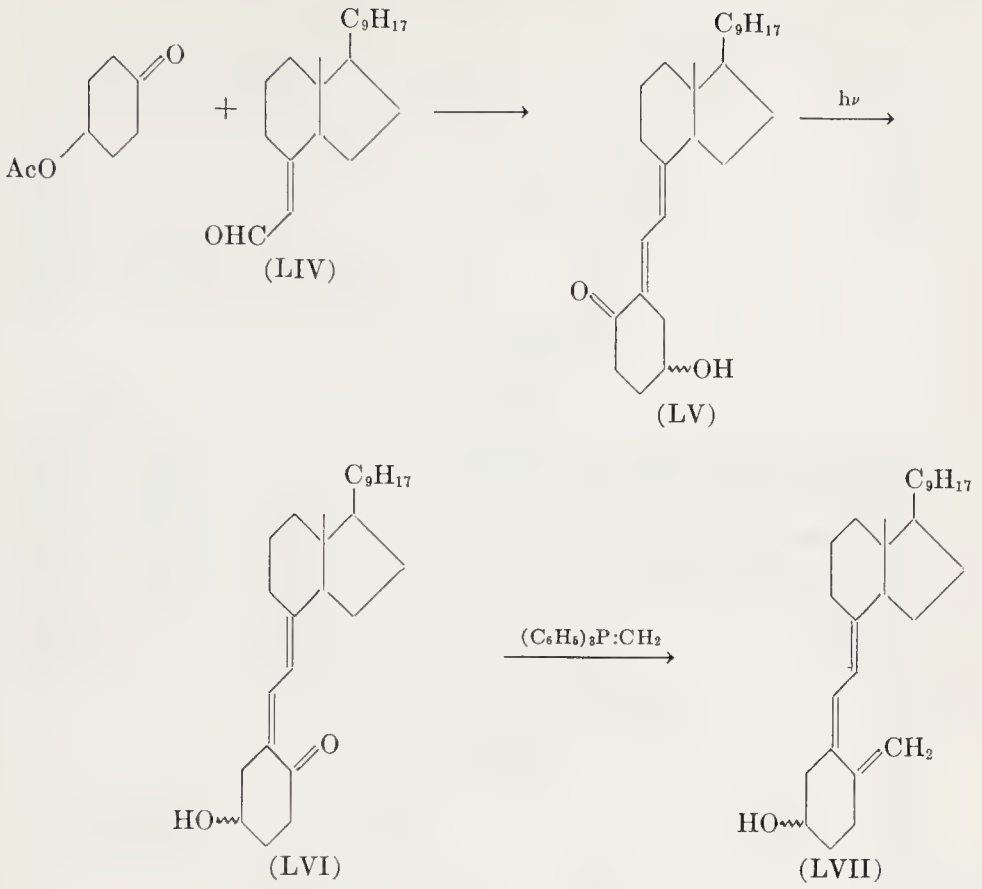


(LIII)

A recent partial synthesis of calciferol (vitamin D_2) (16) started from the C_{21}-aldehyde (LIV), which condensed with 4-acetoxycyclohexanone to give a mixture of *trans*-dienones (LV) epimeric about C-3. Quantitative ultraviolet isomerization to the 5,6-*cis*-epimer mixture (LVI) followed by reaction with methylenetriphenylphosphorane gave a mixture of calciferol and *epi*-calciferol (LVII), separated as the 3,5-dinitrobenzoates. Treatment of the mixture of *trans*-dienones (LV), as the acetates, with methylenetriphenylphosphorane gave what is probably 5,6-*trans-epi*-calciferol (16,23).

B. POLYENES

Several polyenes have been synthesized for use in light-absorption studies, among them the series $Me(CH:CH)_nMe$ where n = 8, 9, and

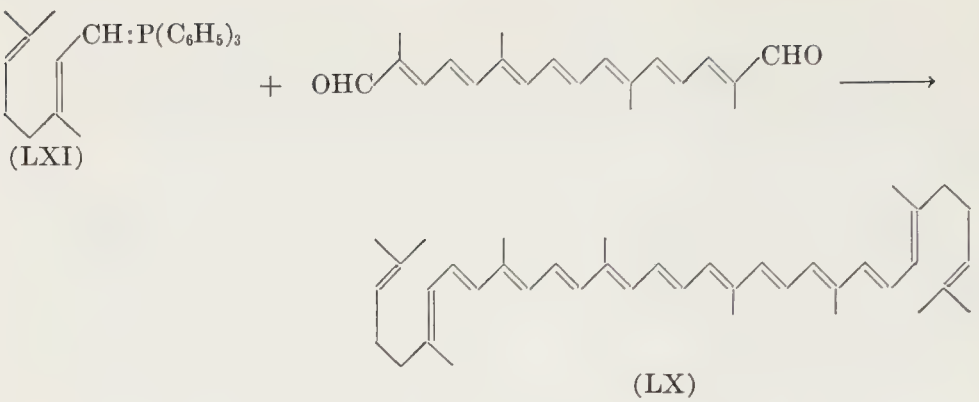


10 (9). The decaene was obtained by reaction of the dialdehyde (LVIII) with *n*-hexa-2,4-dienylidenetriphenylphosphorane (LIX) and partial hydrogenation of the resulting diacetylene.

$$\underset{\text{(LIX)}}{Me(CH{:}CH)_2CH{:}P(C_6H_5)_3} + \underset{\text{(LVIII)}}{CHOCH{:}CH(C{\vdots}C)_2CH{:}CHCHO} \longrightarrow$$

$$Me(CH{:}CH)_4(C{\vdots}C)_2(CH{:}CH)_4Me \longrightarrow Me(CH{:}CH)_{10}Me$$

In the carotenoid field, besides vitamin A (see Section III-A), lycopene (26), 15,15′-dehydrolycopene (26), 15,15′-*cis*-lycopene (26), 15,15′-dehydro-β-carotene (26), and norbixin and crocetin diesters (27) have been synthesized using the Wittig reaction. A typical synthesis is that of lycopene (LX), in which the phosphorane (LXI), derived from geranyl bromide, was treated with crocetin dialdehyde in methylene dichloride solution.



(LXI) (LX)

Among the naturally occurring polyenes, cicutol (LXII) (12), the phenyldienetriyne (LXIII), and the polyene (LXIV) obtained from the *Coreopsis* species (3), oenanthetol (LXV) (11), the polyacetylene hydrocarbon (LXVI) obtained from *Artemesia vulgaris* (7), the insecticide anacycline (LXVII) (6), and an all-*trans* isomer of the polyacetylene (LXVIII) obtained from *Carlina vulgaris* (5), have been synthesized using the Wittig reaction.

$$n\text{-}C_4H_9(CH{:}CH)_3(C{:}C)_2CH_2CH_2CH_2OH$$
(LXII)

$$C_6H_5(C{:}C)_3(CH{:}CH)_2Me$$
(LXIII)

$$MeCH{:}CH(C{:}C)_2(CH{:}CH)_3H$$
(LXIV)

$$n\text{-}C_6H_{13}(CH{:}CH)_2(C{:}C)_2CH{:}CHCH_2OH$$
(LXV)

$$n\text{-}C_3H_7(C{:}C)_2CH_2CH_2(CH{:}CH)_2CONHCHMe_2$$
(LXVI)

$$Me(C{:}C)_3(CH{:}CH)_2(CH_2)_4CH{:}CH_2$$
(LXVII)

$$CH_2{:}CHCH{:}CH(C{:}C)_3CH{:}CHCH_2OCOMe$$
(LXVIII)

In the synthesis of anacyline, the phosphorane (LXIX) was added to the ethyl ester of fumaraldehydic acid; that is, reverse addition was used to prevent excess phosphorane reacting with the ester group of the aldehyde-ester.

$$n\text{-}C_3H_7(C{:}C)_2CH_2CH_2CH{:}P(C_6H_5)_3 + CHOCH{:}CHCOOEt \longrightarrow$$
(LXIX)

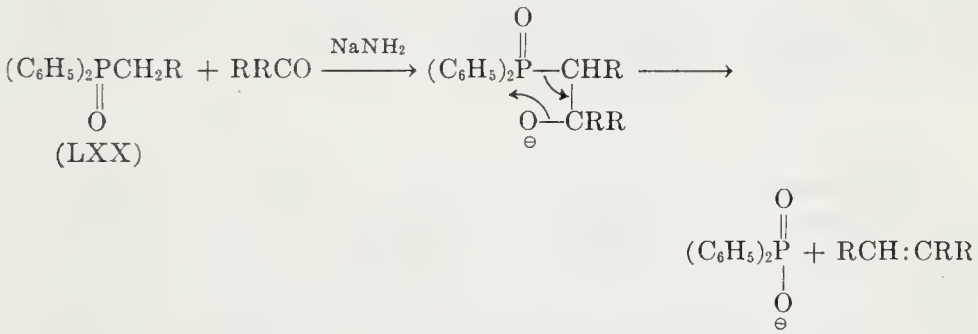
$$n\text{-}C_3H_7(C{:}C)_2CH_2CH_2(CH{:}CH)_2COOEt$$

C. MISCELLANEOUS

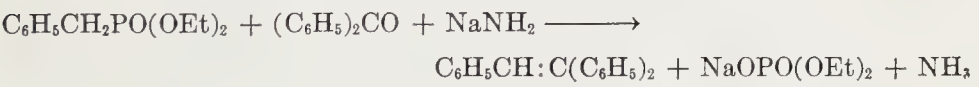
A recently described aldehyde synthesis (28) involving triphenyl (methoxymethyl)phosphonium chloride, prepared from triphenylphosphine and chloromethyl methyl ether, illustrates an application of the Wittig reaction to the preparation of nonolefinic products. Further applications of the principle of the Wittig reaction to organic synthesis may be anticipated.

V. Related Reactions

Horner (42) has recently described two olefin syntheses analogous to the Wittig reaction. In the first of these, an alkyldiphenylphosphine oxide (LXX), on refluxing in benzene with a ketone and sodium amide, gives sodium diphenylphosphinate and an olefin; e.g., methyldiphenylphosphine oxide with benzophenone and sodium amide in benzene under reflux for 4 hr. gave 1,1-diphenylethylene (70%) and sodium diphenylphosphinate (96%).



In the second olefin synthesis, diethyl benzylphosphonate on refluxing with benzophenone and sodium amide in benzene for 6 hr. gave triphenylethylene (88%).



VI. Experimental

A. PREPARATION OF ALKYLTRIPHENYLPHOSPHONIUM HALIDES

Alkyltriphenylphosphonium halides are prepared by the action of alkyl halides on triphenylphosphine. With allyl halides and

the lower alkyl halides, the reaction proceeds readily, either at room temperature or on gentle heating in a solvent such as benzene. With increasing size of the alkyl radical, more vigorous conditions are required, and the reactants may be heated together without solvent, or refluxed in a polar solvent such as nitromethane. The order of reactivity of alkyl halides is RI > RBr > RCl.

The resulting phosphonium salts are in general crystalline solids. They show a tendency to adsorb water and should always be carefully dried before use. The crystallization of phosphonium halides from complex mixtures is often difficult, and tetrahydrofuran has proved to be a useful solvent in this connection (15). Experience has shown that the Wittig reaction can be carried out successfully only with pure crystalline phosphonium halides.

Methyltriphenylphosphonium Bromide (41). To a solution of triphenylphosphine (100 g.) in benzene (500 ml.) contained in a pressure flask at −10°, methyl bromide (50 g.) was added, and the flask set aside at room temperature for 2 days. The resulting methyltriphenylphosphonium bromide (101 g.), m.p. 227–229°, was then filtered and washed with benzene.

B. THE WITTIG REACTION

In the original procedure for carrying out the Wittig reaction (41), an alkyltriphenylphosphonium bromide, suspended in ether under oxygen-free nitrogen at room temperature, was treated with phenyllithium (1 mole) in ether, the aldehyde or ketone then added, and the resulting betaine (VI) decomposed by heating the suspension in a Schlenk tube at 65° for 2–3 hr. Triphenylphosphine oxide and lithium bromide formed an ether-insoluble complex which was then removed by filtration.

To avoid use of a Schlenk tube, ether may be replaced by tetrahydrofuran (b.p. 64°) (17). This solvent is particularly useful where one component of the reaction contains a free hydroxyl group which may lead to the formation of ether-insoluble lithium complexes (37), but suffers from the serious drawback that the triphenylphosphine oxide–lithium bromide complex is soluble, and the product must therefore be separated from an equimolecular quantity of triphenylphosphine oxide. In many cases this separation is not readily achieved; triphenylphosphine oxide shows a tendency to leak slowly through all but the most carefully conducted chromatography columns.

The use of heat is unnecessary for the decomposition of many intermediate zwitterions, particularly when a conjugated system of double bonds is being set up or extended. Thus, the phosphorane (LXXI) with pent-2-ene-4-ynal in ether gave a 60% yield of the triene-yne (LXXII) after only 1 hr. at room temperature.

$$n\text{-}C_4H_9CH{:}CHCH{:}P(C_6H_5)_3 + CHOCH{:}CHC{\vdots}CH \longrightarrow$$
(LXXI)

$$n\text{-}C_4H_9(CH{:}CH)_3C{\vdots}CH$$
(LXXII)

n-Butyllithium is often used instead of phenyllithium in the preparation of phosphoranes (13), but appears to have little advantage over the latter more stable reagent. Triphenylmethylsodium (13), powdered sodium in ether (32), molten potassium (29), ethylmagnesium bromide in tetrahydrofuran (15), and sodamide in xylene (40) have also been used. Treatment of an alkyltriphenylphosphonium halide in ethanol with sodium ethoxide gives an equilibrium mixture of alkyltriphenylphosphonium ethoxide and the corresponding phosphorane (40) which may be used directly in the Wittig reaction.

$$(C_6H_5)_3\overset{\oplus}{P}CH_2R\ \overset{\ominus}{O}Et \rightleftharpoons (C_6H_5)_3P{:}CHR + EtOH$$

Benzyltriphenylphosphonium chloride with sodium ethoxide in ethanol and benzaldehyde at room temperature gave a 76% yield of stilbenes and 20% of unchanged phosphonium halide (39). Sodium alkoxides have found particular use with phosphonium halides derived from α-bromoesters, but recent work has shown that these stable phosphoranes may in general be prepared in aqueous solution using sodium hydroxide as the base (27).

3-Methylenecholestane (1). Methyltriphenylphosphonium bromide (2.77 g.) was treated with phenyllithium (651 mg.) in ether (46 ml.) with shaking for 3 hr. Cholestanone (3.0 g.) was then added, and the solution was refluxed overnight. After being washed with water, the ethereal solution was dried and evaporated *in vacuo*. The residue was refluxed with excess lithium aluminum hydride in ether. After being worked up in the usual way, the product was filtered in light petroleum through alumina (50 g.). Elution with the same solvent (150 ml.) afforded 3-methylenecholestane (1.6 g.) as needles (from ethyl acetate–methanol), m.p. 65–66°, $[\alpha]_D$ +23° (*c* 2.16 in CCl_4).

Dicyclohexylideneethane (17). Cyclohexylideneëthyltriphenyl-

phosphonium bromide (10 g.), suspended in dry ether (100 ml.), was treated under oxygen-free nitrogen with 1.3*N* ethereal *n*-butyllithium (21.5 ml.); the solid rapidly dissolved and the red solution was kept overnight at room temperature. After the addition of cyclohexanone (2.56 g.) in ether (15 ml.), the solution was stirred for 2 hr., and the ether was removed by distillation and replaced by tetrahydrofuran. The resulting solution was heated under reflux for 2 hr., and the tetrahydrofuran removed under reduced pressure. The residue was extracted with ether and the ethereal solution washed with water, dried, and evaporated. Distillation of the residue gave dicyclohexylideneethane (3 g.), b.p. 85–87°/0.1 mm., which separated from light petroleum (b.p. 40–60°) as colorless crystals, m.p. 45–47°.

Carbomethoxymethylenetriphenylphosphorane (27). Carbomethoxymethyltriphenylphosphonium bromide (42.9 g.) was dissolved in water (1 liter) and dilute sodium hydroxide added with stirring until the solution was alkaline to phenolphthalein. The phosphorane was then filtered, dried on a porous tile, and recrystallized from hot ethyl acetate by the addition of light petroleum (b.p. 40–50°). It formed colorless leaves, m.p. 162–163°.

Methylbixin (27). 4,9-Dimethyldodecapenta-2,4,6,8,10-ene-1,12-dial (1.08 g.) and α-carbomethoxyethylidenetriphenylphosphorane (4.4 g.) were dissolved in benzene (50 ml.) and the solution heated under reflux for 6 hr. in a nitrogen atmosphere. On cooling, methylbixin (1.2 g.) slowly crystallized, and a further 0.8 g. was obtained from the mother liquors by evaporation and extraction of the residue with methanol. Recrystallization from benzene yielded pure methylbixin, m.p. 203°, undepressed by natural stable methylbixin.

cis- and trans-Stilbene (40). To a solution of sodium (0.46 g.) in ethanol (50 ml.), benzyltriphenylphosphonium chloride (9 g.) was added under nitrogen and, to the resulting deep yellow solution, freshly distilled benzaldehyde (2.1 g.) was then added. After 54 hr. the solution was poured into dilute hydrobromic acid (50 ml.), and the resulting *trans*-stilbene, m.p. 120.5–121.5°, (1.3 g., 35%) was filtered.

The filtrate was concentrated under a 40 cm. column, extracted with ether, and the extract washed with sodium hydrogen sulfite solution and evaporated. Digestion of the residue with light petroleum gave triphenylphosphine oxide, m.p. 151.5–152.5° (4.2 g., 75%), and distillation of the petroleum extract gave *cis*-stilbene, b.p.

139–140.5°/11 mm., n_D^{20} 1.6200 (1.4 g., 40.5%). Further concentration of the hydrobromic acid aqueous phase gave benzyltriphenylphosphonium bromide, m.p. 277–279° (1.7 g., 20%).

VII. Conclusion

The Wittig reaction is an excellent olefin synthesis, *when substituents present in both the phosphorane and the ketone allow it to be used*, but it is obvious from the above discussion that the great reactivity of most phosphoranes imposes a severe limitation on future use, particularly if the phosphorane is pre-formed using a powerful base such as *n*-butyllithium. It seems likely, however, that formation of the phosphorane *in situ*, using a base such as sodium ethoxide, will be more widely adopted and may overcome this difficulty. In this way it should be possible to prepare and use *in situ* Wittig reagents containing substituents less reactive than carbonyls but which normally react with *n*-butyllithium and with phosphoranes, e.g., the phosphoranes derived from halogenoesters. An extension of this would be use of the Wittig reaction in the preparation of cyclic olefins by treatment with sodium ethoxide of the phosphonium halides derived from halogenoketones.

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HYDROXYLATION METHODS

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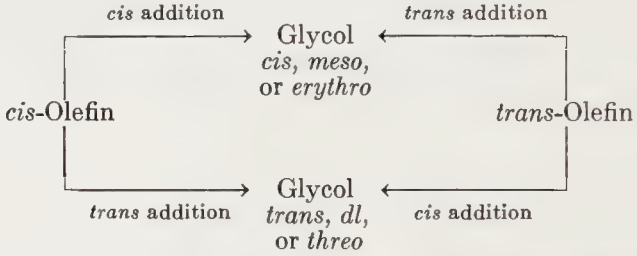
I. Introduction

The partial oxidation of an olefin into the corresponding dihydroxy compound (glycol or α-diol) is known generally by the term hydroxylation.

$$\rangle C{:}C\langle \xrightarrow{\text{hydroxylation}} \rangle C(OH)\cdot C(OH)\langle$$

This reaction has been of value in degradative studies of natural products, in synthetic procedures, and in the characterization of many olefinic compounds; also it has theoretical significance.

There are many reagents which can effect this change, but only the more important will be discussed here. To be of value, hydroxylating reagents must be stereospecific, leading entirely, or at least predominantly, to the *cis* or *trans* addition of the two hydroxyl groups. When the reaction occurs in several stages, the descriptions *cis* and *trans* refer, unless otherwise stated, to the over-all reaction and not to a single stage in the process. The relation between the olefin, which may have a *cis* or *trans* configuration, and the resulting glycol, which may be described by the terms *cis* or *trans*, *erythro* or *threo*, or *meso* or *dl*, depending on the nature of the other groups attached to the glycol system, is set out below.



The chief methods of effecting *cis*-hydroxylation are by reaction with potassium permanganate, with osmium tetroxide alone or as a catalyst, or with silver iodoacetate according to Woodward's procedure. All these have proved useful, though the first two suffer from certain disadvantages; the last method was first reported in 1953 but is being used increasingly. There is evidence that with more complex molecules these reagents are not wholly interchangeable (see Section IV-A).

The most important method of *trans*-hydroxylation is undoubtedly the reaction with peracids, though the Prévost reaction and oxidation with hydrogen peroxide in alkaline solution or in the presence of certain oxide catalysts are also useful procedures.

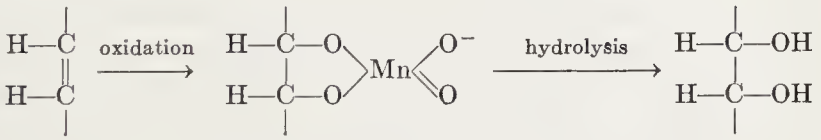
Other hydroxylation methods not discussed here because of their limited value or because of their indirectness include: (*a*) addition of halogens and subsequent hydrolysis of the dihalide; (*b*) addition of hypothalous acid and subsequent hydrolysis of the halogenohydrin;

(*c*) reaction with lead tetraacetate in hot acetic acid solution, (*d*) reaction with mercuric acetate; and (*e*) reaction with chromic acid.

II. Potassium Permanganate

Oxidation by potassium permanganate is one of the simplest and earliest procedures for effecting hydroxylation of olefins and is still widely used despite its limitations. Because this reagent can oxidize olefins in several other ways, and because the desired glycol may be subject to further oxidation or to acid- or alkali-catalyzed isomerization, it is necessary to control the reaction conditions carefully if the yield of glycol is not to be considerably reduced by extensive side reactions. The best results are usually obtained in alkaline media using water or aqueous organic solvents; other oxidation products may accompany or replace the diol in neutral or acid media.

Though it is well known that oxidation by permanganate is a *cis*-hydroxylation, the detailed mechanism of the reaction is less clear. The scheme involving a cyclic complex of manganese was first elaborated by Böeseken (12) and is attractive because Criegee (27) subsequently showed that *cis*-hydroxylation by osmium tetroxide occurs through a structurally similar cyclic compound (Section III-A). This view has recently found acceptance (73,105); it is considered that the cyclic intermediate (I) is formed first and is subsequently hydrolyzed to the *cis*-glycol



(I)

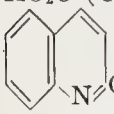
Wiberg and Saegebarth (150), using permanganate labeled with O^{18}, have shown that both glycol oxygen atoms come from the oxidizing agent. They have further suggested how the intermediate (I) might react under different conditions to furnish a ketol or fission products (Section II-B).

A. OXIDATION IN ALKALINE SOLUTION

In Aqueous Solution. In the general procedure, the olefin, dissolved in dilute sodium carbonate or hydroxide solution, is treated with a slight excess of dilute permanganate solution at room temperature or below. When reaction is complete, the precipitated manganese

dioxide is removed by filtration or dissolved by treatment with sulfur dioxide. The glycol then frequently separates from the aqueous solution or is extracted with an appropriate solvent. The conversion of insoluble manganese dioxide to a soluble manganese salt is probably to be preferred, especially with acids which are reported to be strongly adsorbed by manganese dioxide (91). This method is particularly suited for the hydroxylation of olefinic acids, since these are soluble in aqueous alkali and the oxidation product usually precipitates when the solution is finally acidified. Typical olefins which have been hydroxylated in this way, and the yields, are listed in Table I.

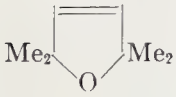
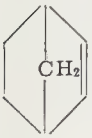
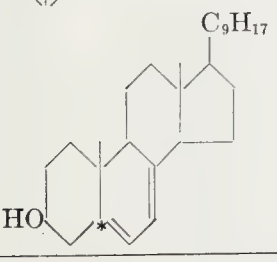
TABLE I
Typical Olefins Hydroxylated by $KMnO_4$ in Aqueous Alkaline Solution

Olefin	Glycol yield, %	Ref.
$CH_2{:}CH{\cdot}CH(OEt)_2$	67	157
$CH_3{\cdot}(CH_2)_7{\cdot}CH{:}CH{\cdot}(CH_2)_7{\cdot}CO_2H$	96	74
	70–75	143
$HO_2C{\cdot}(CH_2)_7{\cdot}CH{:}CH{\cdot}(CH_2)_7{\cdot}CO_2H$	81	33
	?	38

Lapworth and Mottram (74), after a particularly careful study of the oxidation of oleic acid to 9,10-dihydroxystearic acid, describe an experimental procedure for estimating oleic acid by this reaction. They claim almost quantitative yields when the following conditions are observed: (*a*) the reaction must be effected at 0–10°, (*b*) the concentration of oleic acid as its sodium or potassium salt should not exceed 0.1%, (*c*) the concentration of permanganate solution should not exceed 1%, (*d*) only a short reaction period of about 5 min. is required, (*e*) a slight excess of alkali should be present from the beginning to prevent the formation of ketohydroxystearic acid (Section II-B). Though no longer used for this quantitative purpose, this procedure is nevertheless widely employed in the qualitative identification of oleic and other monoolefinic acids. It is, however, an unsatisfactory method of preparing large amounts of dihydroxy acids by reason of the large volumes involved; the total reaction mixture is 1 liter of solution per gram of oleic acid. Traynard (143) has modi-

TABLE II

Olefins Oxidized by $KMnO_4$ in Aqueous Solutions of Indicated Solvents

Olefin	Solvent	Glycol yield, %	Ref.
$ClCH_2 \cdot CH:CH \cdot CH_2Cl$	$EtOH/MgSO_4$	49	98
$ClCH_2 \cdot CHCl \cdot CH:CH_2$	$EtOH/MgSO_4$	56	98
$Me_2C:CH \cdot CO \cdot CH_3$	$COMe_2$	60	56
$MeO_2C \cdot CH_2 \cdot CH:CH \cdot CH_2 \cdot CO_2Me$	$EtOH/MgSO_4$	28	76
$Ph \cdot CH:CH \cdot CO_2Me$	EtOH	62	120
$p\text{-}NO_2 \cdot C_6H_4 \cdot CH_2 \cdot CH:CH_2$	$COMe_2$	41	42
	$COMe_2$	45	7
	$EtOH/MgSO_4$	34	100a
	t-BuOH	55	150
	$EtOH/MgSO_4$	33	23
	t-BuOH	38	150
	$EtOH/MgSO_4$	35	99
	t-BuOH	38	150
	$EtOH/MgSO_4$	8	61
	$EtOH/MgSO_4$	17	61
	$EtOH/MgSO_4$	24	132
	*t*-BuOH	45	150
	Methylcyclohexane	40	44

* This double bond is oxidized.

fied this procedure to give moderate yields (70–75%) at a dilution of only 40 ml. per gram of olefin.

This reagent affords diglycols with dienes having isolated double bonds (52), but with conjugated dienes the reaction is more complex. Large volumes of water should be avoided when preparing tetra- and hexahydroxy compounds because of their appreciable water solubility.

In Aqueous Organic Solvents. The above procedure is less satisfactory with the more numerous water-insoluble olefins and attempts have been made to oxidize these in aqueous organic solvents. The most popular solvents are acetone and ethanol, though methylcyclohexane and *tert*-butanol have also been used. Ideally, of course, the permanganate should not react with the solvent, a condition satisfied by acetone and *tert*-butanol. Since the oxidation of ethanol by permanganate is a base-catalyzed reaction, it is usual to add magnesium sulfate to the reaction mixture, as the precipitation of insoluble magnesium hydroxide limits the alkalinity of the reaction medium. The olefins listed in Table II have been oxidized by potassium permanganate in aqueous solutions of the stated solvent to furnish the corresponding glycol. The yields are somewhat lower than in the wholly aqueous solution.

B. OXIDATION IN NEUTRAL AND ACIDIC SOLUTION

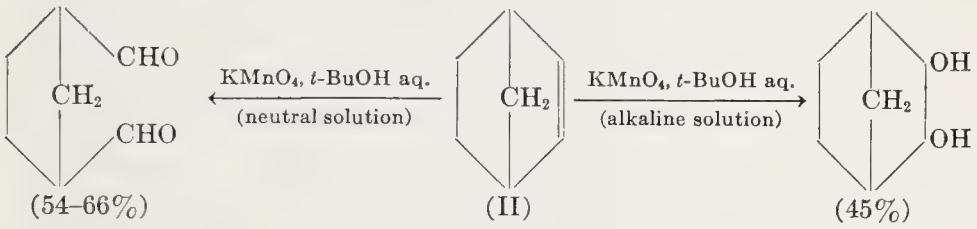
Oxidation of oleic acid by potassium permanganate and insufficient potassium hydroxide is known to yield a mixture of 9,10- and 10,9-ketohydroxystearic acids in addition to the usual diol. Swern *et al.* (24) have shown that with one equivalent of potassium hydroxide the yields of diol and ketol are dependent on the pH of the solution and a high yield of ketol results when the reaction mixture is kept neutral; inexplicably, the same reaction with elaidic acid (the *trans*

TABLE III
Effect of pH on Products of Permanganate Oxidation

	Oleic acid		Elaidic acid	
pH at end of experiment	Diol, %	Ketol, %	Diol, %	Ketol, %
9–9.5	4	75	20	45
11.8	60	20	30	45

isomer of oleic acid) is only slightly pH dependent (see Table III).

Oxidations in aqueous ethanol containing magnesium sulfate do not give high yields of glycol, but no reference has been made to the isolation of ketols or other oxidation products. The oxidation of bicyclo-[2,2,1]-2-heptene (II) with permanganate in aqueous *tert*-butanol, however, is reported to be pH dependent (150), double bond fission occurring in neutral solution



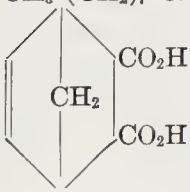
(54–66%) (II) (45%)

Oxidation of some sterols by potassium permanganate in acetic acid solution, under conditions which do not lead to double bond fission, furnishes hydroxy ketones sometimes accompanied by epoxides (37,81).

C. POTASSIUM MANGANATE

Potassium manganate, K_2MnO_4, in alkaline solution at room temperature converts olefins to their *cis*-diols (105,121). The olefins given in Table IV were hydroxylated in this manner, but practically no diol resulted from cyclohexene or hendec-10-enoic acid, though in both cases the oxidizing agent was consumed. So far as present results

TABLE IV
Olefins Hydroxylated in Alkaline K_2MnO_4 Solution

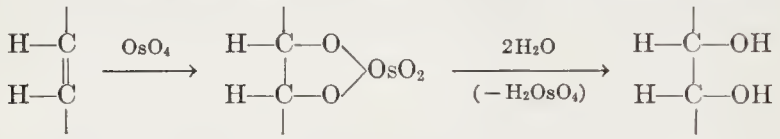
Olefin	Glycol yield, %
$R \cdot CH{:}CH \cdot CO_2H$	
R = Ph	40
R = Me	25
$CH_3 \cdot (CH_2)_7 \cdot CH{:}CH \cdot (CH_2)_7 \cdot CO_2H$	32
	50

indicate, the method seems to have no advantage over the more conventional permanganate procedure.

III. Osmium Tetroxide

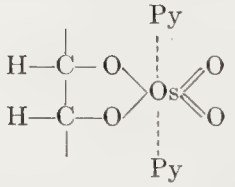
A. REACTION WITH ALIPHATIC AND ALICYCLIC OLEFINS

Although osmium tetroxide had been used previously as a catalyst in hydroxylation procedures, Criegee (27) was the first to show that this compound is itself a most satisfactory hydroxylating agent. When treated at ordinary temperatures with an equivalent quantity of osmium tetroxide in anhydrous ether; or less frequently in benzene (28), cyclohexane (28), or dioxane (8,27) solution, olefins slowly form addition complexes which usually precipitate from the solution in an almost quantitative yield during a period of up to 4 days. This ester complex (III) may be decomposed to osmic acid, or to another osmium derivative, and the diol derived from the original olefin



(III)

This corresponds to a *cis* addition of hydroxyl groups and is a valuable *cis*-hydroxylation procedure. The initial addition of osmium tetroxide is accelerated by tertiary bases, especially pyridine (28). Bright-colored complexes (IV) in which osmium is coordinated with



(IV)

two molecules of base separate in almost quantitative yield and these may be similarly decomposed to a diol. Various reagents have been used to decompose the osmium derivatives (III) and (IV), including (*a*) acidic solutions of sodium or potassium chlorate (27,114), (*b*) sodium sulfite in aqueous ethanol (20,27,127,151,152), (*c*) alkaline solutions of mannitol (28,108,110) or formaldehyde (118), and (*d*) hydrogen sulfide (8). Sodium sulfite and mannitol seem to be the most popular.

Despite the practical simplicity of this reaction and the high yields which result from it, the method suffers from certain disadvantages associated with the properties of osmium tetroxide. This reagent is very expensive and has a high equivalent; it is fairly volatile and its vapors are very poisonous and seriously injure the eyes. For these reasons, it is unsuited for large-scale preparations, and its use has been largely restricted to the study of scarce materials. By reason of the smoothness of the reaction it has, nevertheless, been an invaluable aid in the investigation of substances available in limited quantitites, e.g., steroid hormones (129).

The *cis*-glycols listed in Table V are typical substances prepared by this means from the corresponding olefin. Yields are quoted, for the formation of the osmic ester (III) or (IV) and the derived *cis*-glycol, respectively, or for the over-all yield from conversion of olefin to diol. Further examples are given in refs. 8. 20, 114, and 118.

B. REACTION WITH AROMATIC COMPOUNDS

Cook and Schoental (25) and Badger (4,5), following Criegee's observation (28) that osmium tetroxide is able to hydroxylate phenanthrene in the 9,10-position, have studied the effect of this reagent on other polycyclic aromatic hydrocarbons containing the phenanthrene system, and on anthracene. Reaction is slower than with ethylenic compounds and only the most reactive aromatic *bonds* are attacked. This contrasts with the attack by ionic reagents at the most reactive *centers* in the molecule and has theoretical significance in the study of double-bond character in polycyclic compounds (4,5). These results are of enhanced interest because the products resemble those formed by metabolic oxidation of these hydrocarbons (25). The glycols in Table VI have been obtained from the indicated compounds (4,5,25,72).

C. OXIDATION BY OSMIUM TETROXIDE AND METAL CHLORATES

Osmium tetroxide has also been used as a catalyst in hydroxylation procedures. K. A. Hofmann (63) first reported that aqueous solutions of sodium or potassium chlorate, on addition of a little osmium tetroxide, become strong oxidizing agents able to hydroxylate ethylenic centers and to exert other oxidizing functions. Braun (16a) found that the yields increased with the use of barium or silver chlorate, and these have been widely used by subsequent investi-

TABLE V
Typical *cis*-Glycols Prepared by OsO_4 Hydroxylation of Olefins

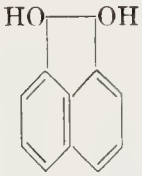
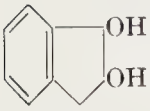
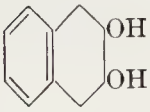
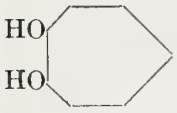
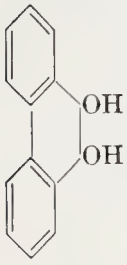
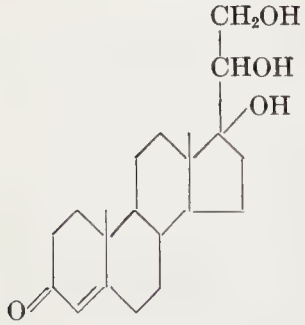
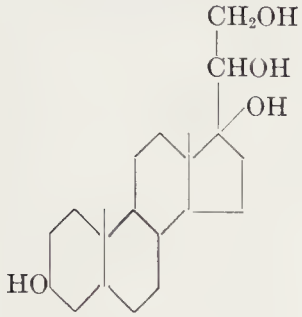
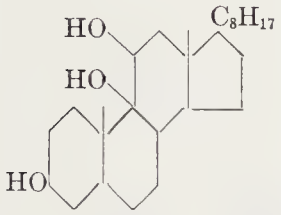
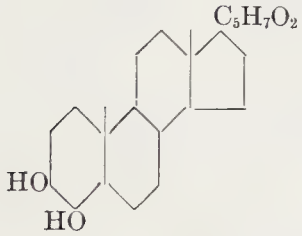
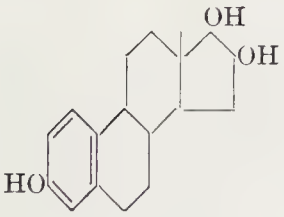
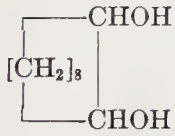
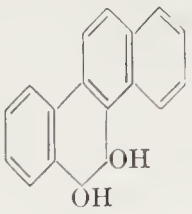
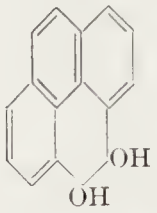
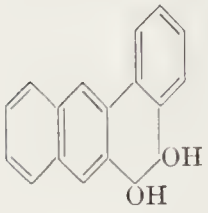
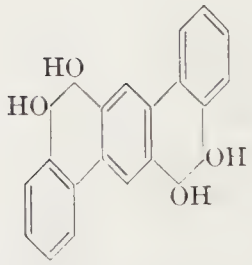
cis-Glycol	Yield, %			Ref.
	Osmic ester (III) or (IV)	Derived *cis*-glycol	Diol	
	98	84		27
	99	66		27
	89	78		27
	96	81		27
	98	100		28
	95	64		28
			60	127

TABLE V (*continued*)

cis-Glycol	Yield, %		Diol	Ref.
	Osmic ester (III) or (IV)	Derived *cis*-glycol		
			?	128
			60	151
			100	152
			80	108
			87	110

gators. Reaction takes place in aqueous solution at temperatures between 0 and 50°, sometimes occurring slowly during several days or

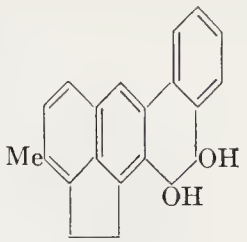
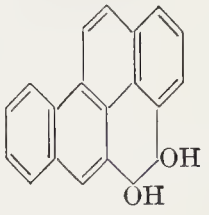
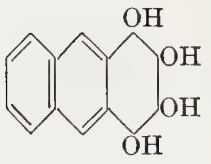
TABLE VI
Glycols Obtained by OsO_4 Hydroxylation of Aromatic Hydrocarbons

Starting compound	Glycol
Chrysene	
Pyrene	
1,2-Benzanthracene[a]	
1,2,5,6-Dibenzanthracene	

even weeks, and is most satisfactory with compounds having some degree of water solubility. Within these limitations, however, the procedure works well and has been used particularly for the oxidation of shorter chain aliphatic olefins, as, for example, those listed in Table VII, for which the yield and chlorate used are given. In addition to these and to the earlier experiments of Hofmann (63,64), this procedure has been used to oxidize 1,2-dihydroxycyclohex-3-ene (107) and in Lespieau's preparation of hexitols and pentitols from unsaturated polyhydric alcohols (75).

The products are clearly those resulting from *cis*-hydroxylation, and it has been suggested that reaction occurs by addition of osmium

TABLE VI (*continued*)

Starting compound	Glycol
20-Methylcholanthrene	
3,4-Benzpyrene	
Anthracene[b]	

[a] The 1′-methyl-, 9-methyl-, 9,10-dimethyl-, 5,9,10-trimethyl-, and 5,6,9,10-tetramethyl-1,2-benzanthracenes behave similarly.

[b] The formation of a tetrol from anthracene is conceived as follows: the initial product, formed by addition to the 1,2-bond, contains a true ethylenic bond (in the 3,4-position) which readily reacts with a second molecular proportion of reagent.

tetroxide to olefin, decomposition of the osmic ester, and regeneration of the tetroxide by the chlorate (12,27). The view has also been expressed that the reaction involves free hypochlorous acid as a source of hydroxyl radicals (147).

D. OXIDATION BY OSMIUM TETROXIDE, HYDROGEN PEROXIDE, AND *tert*-BUTANOL (MILAS' REAGENT)

Milas and Sussman (88a) prepared anhydrous solutions of hydrogen peroxide in *tert*-butanol (though Mugdan and Young (93) have questioned the necessity for this) and found that such solutions are stable at room temperature for long periods of time and are inert towards olefinic centers. In the presence of a suitable catalyst such as osmium tetroxide, however, the reagent is able to hydroxylate a

TABLE VII
Olefins Oxidized by Chlorate with OsO_4 Catalyst

Olefin	Potassium chlorate		Barium chlorate		Silver chlorate	
	Glycol yield, %	Ref.	Glycol yield, %	Ref.	Glycol yield, %	Ref.
$R \cdot CH_2 \cdot CH{:}CH \cdot CO_2H$						
R = H	17	50	48	16a	83	16a
					66–70	48
R = OH[a]			64	16a	45	49
					75	16a
R = Cl			78	16b		
			75	49		
R = Br			70	16d		
R = Me					80	16c
R = Et					86	16c
$HO_2C \cdot CH{:}CH \cdot CO_2H$ (*cis* and *trans* isomers)	97 (97[b])	90 90				
O:C·CH:CH·C:O (O bridge)			100	16a	70	16a
	(46[b])	23				

[a] Lactone thereof.
[b] Yield with sodium chlorate.

wide variety of olefinic compounds to give *cis*-diols. Reaction occurs at 0° or thereabouts during several hours or a few days. The compounds listed in Table VIII have been so oxidized (88a,88b,89,122) and the conversion of cyclopentadiene to dihydroxycyclopentene has also been studied (87,100b); with certain olefinic sterols this reagent furnishes a mixture of diol and ketol (65,82).

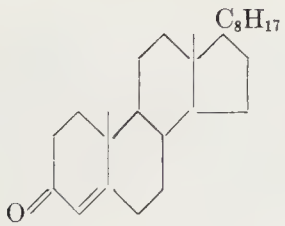
The effect of the Milas reagent on polycyclic aromatic compounds dissolved in acetone has been studied by Cook and Schoental (26). Though phenanthrene and 1,2-benzanthracene afford some *cis*-diol along with quinones and acids resulting from ring fission, other compounds yield only quinones and acidic products. These results differ from those obtained with osmium tetroxide, ether, and pyridine (Section III-B), a point of some interest in view of the opinion that oxidation by the Milas reagent occurs, like the Criegee oxidation, by hydrolysis of a cyclic osmic ester (26,93).

TABLE VIII
Compounds Oxidized by Milas' Reagent

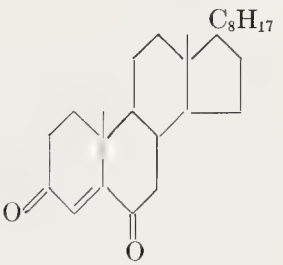
Substance	Glycol yield, %	Substance	Glycol yield, %
Hydrocarbons		Acids and esters	
$CH_2:CH_2$	88–97	$HO_2C\cdot CH:CH\cdot CO_2H$ (*cis*)	30
$MeCH:CH_2$	68	$HO_2C\cdot CH:CH\cdot CO_2H$ (*trans*)	48
$Me_2C:CH_2$	38	$EtO_2C\cdot CH:CH\cdot CO_2Et$ (*cis*)	41
$Me_2C:CHMe$	38–40	$EtO_2C\cdot CH:CH\cdot CO_2Et$ (*trans*)	58
$EtCH:CHMe$	26–30	$CH_3\cdot CH:CH\cdot CO_2H$	54
$EtCH:CHEt$	36	$CH_3\cdot CH:CH\cdot CO_2Et$	56
$EtC(Me):CH_2$	45–51	$Ph\cdot CH:CH\cdot CO_2H$	56
Cyclopentadiene	61[a]	Oleic acid	60
Methylenecyclobutane	39	Alcohols, etc.	
		$CH_2:CH\cdot CH_2OH$	60
Cyclohexene	58	$Ph\cdot CH:CH\cdot CH_2OH$	12
Diallyl	45[a]	$Me_2C:CH\cdot CO\cdot Me$	23
Styrene	50	$Me\cdot CO\cdot O\cdot CH:CH_2$[b]	60[b]
(+)-Limonene	35[a]	$CH_2:CH\cdot O\cdot CH:CH_2$[b]	96[b]
Cetene	77–82		

[a] These compounds afford the tetrol when oxidized.
[b] These compounds afford $CH_2OH\cdot CHO$ when oxidized.

The hydroxylation of some $\alpha\beta$-unsaturated ketones by hydrogen peroxide and osmium tetroxide in other solvents (ether, benzene) has been applied to a number of steroids of which the following are typical:



(Yield: 60%; ref. 21)



(Yield: quantitative; ref. 21)

IV. Halogens and Silver Carboxylates (Woodward, Prévost)

The products of interaction of halogens and the silver salts of carboxylic acids react with olefins, a fact which underlies the Woodward and Prévost methods of *cis*- and *trans*-hydroxylation, respectively.

In the molecular ratio of 1:1, iodine and a silver carboxylate form an acyl hypoiodite (V), but, with a molecular ratio of 1:2, reaction proceeds further and the Simonini complex is formed. The structure of this complex is uncertain but is perhaps best represented by (VI) (67,70; but cf. 9).

$$I_2 \xrightarrow[(-AgI)]{R\cdot CO_2Ag} \underset{(V)}{R\cdot CO_2I} \xrightarrow{R\cdot CO_2Ag} \underset{(VI)}{R\cdot CO_2Ag, R\cdot CO_2I}$$

Prévost (111) showed that the complex formed from silver benzoate reacts with olefins to give dibenzoyl esters of the corresponding glycol. He considered that reaction occurred in two stages via the acylated iodohydrin (VII), and the over-all reaction is now recognized as a *trans* addition. Woodward (159), making use of earlier studies by Winstein and Buckles (154), demonstrated how the conversion of acylated iodohydrin (VII) to acylated glycol (VIII) could be made to proceed with inversion. The over-all reaction is then equivalent to *cis* addition and these reactions may be summarized:

$$\text{—CH:CH—} \xrightarrow[\textit{trans}\text{ addition}]{R\cdot CO_2Ag,\ RCO_2I} \underset{(VII)}{\text{—CHI·CH(O·CO·R)—}} \xrightarrow[\text{Prévost}]{\text{Woodward}\curvearrowright}$$

$$\underset{(VIII)}{\text{—CH(O·CO·R)·CH(O·CO·R)—}} \xrightarrow{\text{hydrolysis}} \text{—CH(OH)·CH(OH)—}$$

(R is usually C_6H_5 in the Prévost reaction and CH_3 in the Woodward procedure)

A. WOODWARD'S *cis*-HYDROXYLATION PROCEDURE

Winstein and Buckles (154) showed that in the conversion of α-acetoxy halides to acylated glycols by silver acetate and acetic acid, reaction occurs with retention of configuration under anhydrous conditions, but with complete inversion when the solvent contains at least a molar proportion of water, as in the example

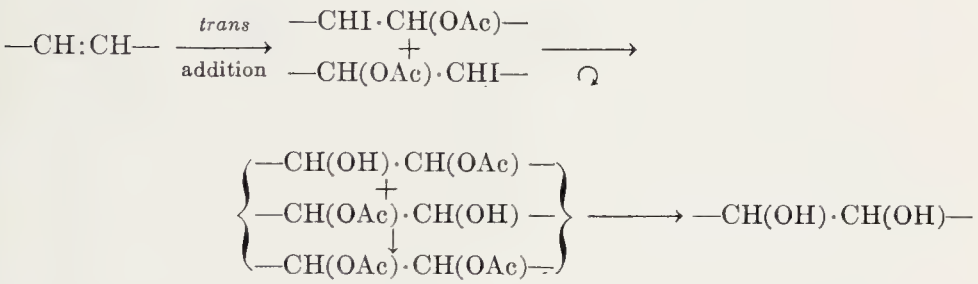
$$\underset{\textit{threo}}{CH_3\cdot CH(OAc)\cdot CHBr\cdot CH_3}$$

$$\downarrow \text{AgOAc, AcOH anhydrous} \qquad \downarrow \text{AgOAc, AcOH aq.}\ \curvearrowright$$

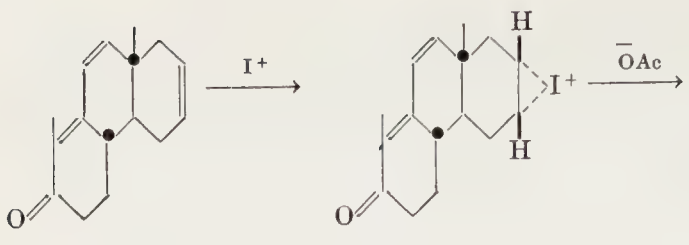
$$\underset{\textit{threo}}{CH_3\cdot CH(OAc)\cdot CH(OAc)\cdot CH_3} \qquad \underset{\textit{erythro}}{CH_3\cdot CH(OAc)\cdot CH(OH)\cdot CH_3}$$

These results were explained by the authors in terms of neighboring group participation by the acetyl group. Woodward and his colleagues (158,159) realized that this result could be used to modify the Prévost reaction so that the initial *trans* addition is followed by a replacement with inversion leading to over-all *cis* addition.

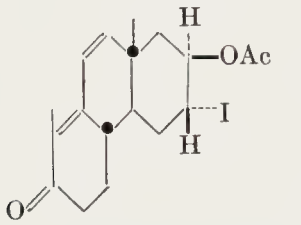
The hydroxylation is effected in three stages (68). Iodine and silver acetate first interact to form a product which converts the olefin to an iodoacetate by *trans* addition. This occurs when the reactants are shaken in dry acetic acid solution at room temperature; the iodine is sometimes added portion-wise, but it is doubtful whether this is necessary. The second stage—replacement of the halogen by a hydroxyl group which may subsequently be acetylated—is effected by silver acetate in acetic acid containing the required amount of water by heating for 3 hr. at 100° or for 1 hr. at the reflux temperature. The mixed mono- and diacetates are finally isolated and hydrolyzed.



This reaction thus provides a method of *cis*-hydroxylation additional to the use of potassium permanganate or of osmium tetroxide and one which does not suffer from the disadvantages associated with these other methods. So far, this procedure has only been used in a limited way, but there is little doubt that it will find increasing use. Its usefulness is enhanced by reports that in more esoteric examples it gives predominantly the opposite *cis* isomer from that resulting with osmium tetroxide. Thus, whereas the *β-cis*-glycols (XI, XII, and XIII) are formed by this reaction, the corresponding *α-cis*-glycols result from oxidation with osmium tetroxide. Woodward and Brutcher (159) explain this result by assuming that the acetylated iodohydrin (X) is formed by attack by I^+ from the least hindered side, followed by reaction with $\bar{O}Ac$:

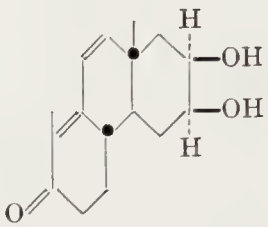


(IX)



(X)

AgOAc, AcOH aq.; hydrolysis



(XI)
(Yield: 71%; ref. 159)
(Yield: 74%; ref. 71)

TABLE IX
Alicyclic Glycols Prepared by Woodward's Procedure

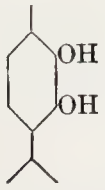
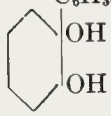
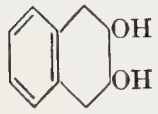
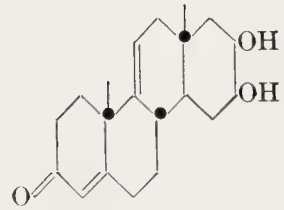
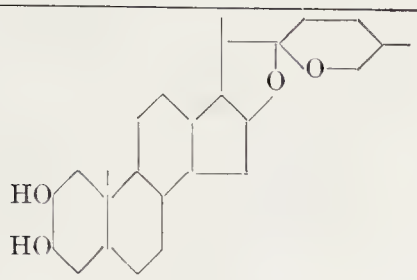
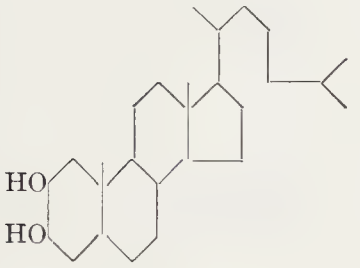
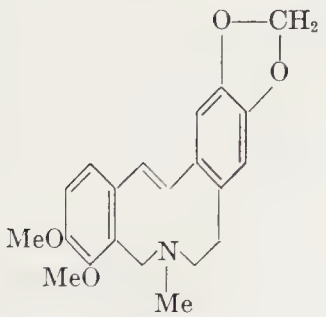
Glycol	Yield, %	Ref.
	73	66
$C_6H_3(OMe)_2$ (2:3) 	56	47
	60	3
	67	6

TABLE IX (*continued*)

Glycol	Yield, %	Ref.
 (XII)	?	32
 (XIII)	?	58
 (XIV)	Nil	125

The method has also been successfully used to prepare the alicyclic glycols listed in Table IX in the over-all yields indicated. The reaction is reported to fail with anhydromethyltetrahydroberberine-A (XIV) (125), possibly because of internal neighboring group participation.

Gunstone and Morris (53) have further shown that this is a very satisfactory means of hydroxylating long-chain ethylenic compounds, and some of their results are summarized in Table X. The preparation of *erythro*-9,10-dihydroxystearic acid directly from olive oil by this method is probably the most convenient route to this compound.

TABLE X
Long-Chain Olefinic Compounds Oxidized by Woodward's Procedure (53)

Olefinic compound	Glycol yield, %	
	Crude	Pure
Pure compounds		
Methyl oleate	99	89
Methyl elaidate	97	91
Elaidic acid	89	85
Oleyl alcohol	100	81
Elaidyl alcohol	94	79
Crude compounds		
Olive oil	87	83[b]
Olive oil[a]	94	97[b]
Castor oil	95	30 + 7[c]
Methyl undecenoate	49	42
Methyl hexadecenoate	93	62
Methyl linoleate	95	15 + 14[d]
Oleic acid	95	56

[a] All oxidations were effected on 0.01 mole of olefinic compound except in this case where 0.1 mole was used.

[b] These values are corrected on the assumption that olive oil contains 75% oleic acid.

[c] These refer to the lower- and higher-melting isomers, respectively, and are corrected on the assumption that castor oil contains 90% ricinoleic acid.

[d] These refer to the lower- and higher-melting tetrahydroxystearic acids, respectively.

By oxidizing erucic acid with iodine and silver acetate, Raman (115) has obtained the *erythro*-glycol (70%) in aqueous acetic acid solution, whereas the *threo* isomer (25%) preponderates in anhydrous acetic acid solution.

Morris (92) has described the *cis*-hydroxylation of methyl oleate (68%) and methyl elaidate with iodine and silver nitrate in wet or dry acetonitrile solution.

B. PRÉVOST REACTION

The Prévost reaction, though not so widely used as might be expected, is nevertheless a useful method of effecting the *trans*-hydroxylation of olefins and has given highly satisfactory results in many cases (153).

Glycol dibenzoates are formed when monoolefins (1 mole) are

treated with silver benzoate (2 moles) and iodine (1 mole) in anhydrous benzene solution. Depending on the reactivity of the olefin, reaction occurs at room temperature or during a period of refluxing which may extend to 50 hr. (111a). While it is not necessary to isolate the silver complex (VI), Hershberg (60) has described a convenient method of extracting it from a Soxhlet thimble into a boiling benzene solution of the olefin. The above reagents are those most frequently used, but the iodine may be replaced by chlorine or bromine, the silver benzoate by the acetate, propionate, *n*-butyrate, *m*-nitrobenzoate, or 3,5-dinitrobenzoate, and the benzene by carbon tetrachloride, chlorofrom, or ether. The best yields, however, are claimed with silver benzoate, the glycol dibenzoate crystallizing easily and being readily hydrolyzed.

Many monoolefins and olefinic compounds have been hydroxylated in this way, and typical products obtained from the corresponding olefins in good yield are shown in Table XI.

Diolefins afford the tetrabenzoates when treated with excess ox-

TABLE XI
Products Obtained from Hydroxylation of Olefins by Prévost Reaction

Glycol or dibenzoate	Yield, %	Ref.
$CH_3 \cdot (CH_2)_7 \cdot CH(OH) \cdot CH(OH) \cdot (CH_2)_7 \cdot CO_2H$	69[a]	155
	75[b]	155
$CH_3 \cdot (CH_2)_n \cdot CH(OH) \cdot CH_2OH$		
$n = 13$	33	97
$n = 15$	73	97
$n = 17$	70	97
$Ph \cdot CH_2 \cdot CH(OBz) \cdot CH_2OBz$	85	60
	44	79
	70[c]	77

I

[a] *erythro*-Glycol from methyl elaidate.

[b] *threo*-Glycol from methyl oleate.

[c] Iodination of the activated benzene ring accompanies hydroxylation.

idizing agent and the unsaturated dibenzoate when there is excess olefin as in McCasland and Horswill's experiments with cyclohexa-1,4-diene (79; cf. 3)

The same authors failed to isolate any recognizable products with the conjugated isomer cyclohexa-1,3-diene, though Prévost and Lutz (112) claimed to have prepared butane-1,2,3,4,-tetrabenzoate and but-3-ene-1,2-dibenzoate (accompanied by a very small amount of but-2-ene-1,4-dibenzoate) from butadiene.

$$CH_2{:}CH{\cdot}CH{:}CH_2 \xrightarrow[\text{reagent}]{\text{excess}} CH_2(OBz){\cdot}CH(OBz){\cdot}CH(OBz){\cdot}CH_2OBz\ (60\%)$$

$$CH_2{:}CH{\cdot}CH{:}CH_2 \xrightarrow[\text{olefin}]{\text{excess}} CH_2{:}CH{\cdot}CH(OBz){\cdot}CH_2OBz\ (80\%)$$

As indicated, the Prévost reaction occurs in two stages and in a modified form provides a convenient route to the intermediate acylated halogenohydrin (VII). For this purpose a molar ratio of silver salt to halogen of 1:1 is required, and carbon tetrachloride and chloroform are the favored solvents, further reaction of the halogen compound occurring less readily in these solvents (1,11,36,55,145,146).

V. Aromatic and Aliphatic Peracids

Since the first report of the perbenzoic acid oxidation of olefins by Prileschajew (113), this name has been associated with the epoxidation and hydroxylation of olefins by peracids generally. Peracid oxidation has proved invaluable in many fields of organic chemistry and was reviewed by Swern in 1949 (134a) and in 1953 (134b).

With all peracids the epoxide (XV) is probably formed first and may be isolated under suitable conditions, but in acidic solution the reactive epoxides pass more or less readily into the monoacylated derivative (XVI) of the glycol (XVII)

$$-CH{:}CH- \xrightarrow[\text{addition}]{cis} \underset{\underset{(XV)}{\diagdown\,O\,\diagup}}{-CH\cdot CH-} \xrightarrow{R\cdot CO_2H} -CH(O{\cdot}CO{\cdot}R){\cdot}CH(OH)- \longrightarrow -CH(OH){\cdot}CH(OH)-$$

(XVI) (XVII)

This change may occur spontaneously in the reaction mixture or may be effected subsequently on the isolated epoxide. Epoxidation and hydroxylation under peracid agency are so closely related that it is not possible to discuss one without reference to the other.

Perbenzoic, perphthalic, peracetic, and performic acids have been most widely used in this connection. The first two readily yield epoxides; although the aliphatic peracids normally react further, satisfactory epoxidation procedures have been developed since these peracids are more conveniently prepared and have several advantages for commercial exploitation (34,35,46a,46b). The recently used pertrifluoroacetic acid shows promise of becoming a valuable addition to this list. Isolated reports of the use of periodic (51) and persulfuric acid (95) also exist.

A. MECHANISM AND STEREOCHEMISTRY

Swern (133a) has collated the results of peracid oxidation of a large number of olefinic compounds and has shown that reaction is facilitated by electron-donating groups attached to the olefinic center and inhibited by electron-attracting groups as illustrated by the two series of compounds in Table XII where reaction rates ($k \times 10^3$) are given for epoxidation with peracetic and perbenzoic acids, respectively.

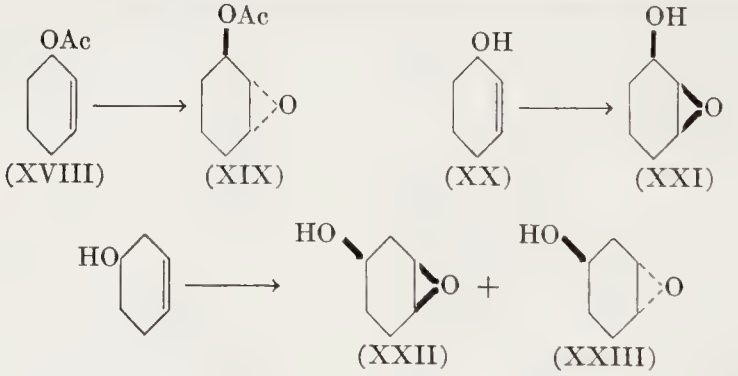
TABLE XII
Reaction Rates for Peracid Oxidation

Olefin	$k \times 10^3$ (peracetic)	Olefin	$k \times 10^3$ (perbenzoic)
$CH_2{:}CH_2$	0.19	$Ph{\cdot}CH{:}CH{\cdot}CH_2OH$	203
$CH_3{\cdot}CH{:}CH_2$	4.2	$Ph{\cdot}CH{:}CH{\cdot}CHO$	4.7
$CH_3{\cdot}CH{:}CH{\cdot}CH_3$	93	$Ph{\cdot}CH{:}CH{\cdot}CO_2H$	0.13
$(CH_3)_2C{:}CH_2$	92		
$(CH_3)_2C{:}CH{\cdot}CH_3$	ca. 1000		
$(CH_3)_2C{:}C(CH_3)_2$	Too high to be measured		

The results are indicative of reaction between nucleophilic olefin and electrophilic peracid and account for the general difficulty of oxidizing in this way Δ^1-olefins and α,β-unsaturated acids and esters (41,135, 136). These less reactive compounds have, nevertheless, been

epoxidized and hydroxylated, most satisfactorily by pertrifluoroacetic acid. Some uncertainty remains concerning the detailed mechanism of this reaction (78,134a,148). Its stereochemistry, on the other hand, seems to be fairly clear (133b). Epoxidation is a *cis* addition, but, as the normal methods of opening the epoxide ring are accompanied by Walden inversion, the over-all change of olefin to glycol is equivalent to *trans* addition. This has been confirmed by a large number of examples. Bharucha and Gunstone (10; cf. 154) have described a method of converting the epoxide into the glycol with two inversions, i.e., over-all retention of configuration.

From a study of the reaction of cyclic olefins with perbenzoic acid, Henbest and his colleagues (57,59) conclude that the reagent usually approaches from the less hindered side of the molecule except in the presence of an allyl hydroxyl group. The acetate (XVIII) thus gives mainly XIX with a *trans* relation between acetoxy and epoxy groups, while cyclohex-2-enol (XX) affords the epoxy alcohol (XXI) having a *cis* relation between the hydroxyl and epoxy groups; cyclohex-3-enol forms a mixture of the two epoxides, XXII and XXIII



(XVIII) (XIX) (XX) (XXI) (XXII) (XXIII)

The reaction velocities show that an allylic hydroxyl group exerts a promoting influence in comparison with its acyl (O·CO·R) and alkyl (OR) derivatives, and it is suggested that hydrogen bonding causes an association of the reactants favorable for interaction between the electrophilic peracid oxygen and the olefin.

B. AROMATIC PERACIDS

This reaction was first discovered with perbenzoic acid (113) which has since been used extensively for the epoxidation of olefins. Reaction proceeds so readily in most cases that it has been suggested as a

means of detecting and estimating olefinic unsaturation (106). Epoxidation is effected at room temperature or below during several hours using chloroform, ether, benzene, acetone, or dioxane as solvent. Details of compounds so oxidized have been collated by Swern (134). Perbenzoic acid is usually prepared by reaction of benzoyl peroxide with sodium methoxide (134b) or ethoxide (141), or of benzoyl chloride with sodium peroxide (17) or hydrogen peroxide (69). It is also formed in the atmospheric oxidation of benzaldehyde, and this has been utilized in a novel epoxidation procedure consisting of cooxidation of benzaldehyde and olefin (138,139).

Monoperphthalic acid, produced by reaction of phthalic anhydride and hydrogen peroxide in alkaline solution (13,14), has also been used for the epoxidation of several olefins (15,134). It is said to have several advantages over perbenzoic acid; in particular, it is more stable, which is an advantage in reactions which proceed very slowly, and phthalic acid, being more insoluble in chloroform than is benzoic acid, is more easily removed. Its method of use is very similar to that of perbenzoic acid.

Use has also been made of percamphoric (84,86a) and perfuroic (86b,86c) acids, but these have no advantage over the more familiar perbenzoic and perphthalic acids.

C. ALIPHATIC PERACIDS

Though many aliphatic peracids are known and some have been used as epoxidizing agents (101a,101b,119) only performic, peracetic, and pertrifluoroacetic acid merit discussion here.

Aliphatic peracids are most conveniently prepared by interaction of the carboxylic acid with hydrogen peroxide when the following equilibrium is established, the peracid reacting quickly with any olefin which may be added to the system (29,30)

$$R \cdot CO_2H + H_2O_2 \rightleftharpoons R \cdot CO_3H + H_2O$$

A method of preparing pure peracetic acid based on the oxidation of acetaldehyde has been described (103).

Peracetic Acid. With acetic acid this equilibrium is attained only slowly, and the reaction of olefins with acetic acid and hydrogen peroxide takes place over several hours or days during which time extensive decomposition of the hydrogen peroxide may occur (31,62). In the presence of a catalyst such as sulfuric acid, however, equi-

librium is reached in about 10 hr. at 25°, and this provides a more convenient method of obtaining peracetic acid. The equilibrium constant is such that with equimolecular quantities of acetic acid and 50% hydrogen peroxide, 40% of the peroxide is converted to peracetic acid, and with molar ratios of 2:1 and 10:1 this value is raised to 57 and 90%, respectively. A large excess of acetic acid is thus required for the economical utilization of the hydrogen peroxide.

Peracetic acid solutions so prepared provide one of the best reagents for hydroxylation of olefinic centers, for the epoxide first formed is readily hydrolyzed to the hydroxy acetate (XVI, R = CH_3), alkaline hydrolysis of which affords the diol. Pre-formed peracetic acid is not required; it is sufficient to maintain mixtures of olefin, acetic acid, hydrogen peroxide, and catalyst at 40°. The utilization of hydrogen peroxide is then almost quantitative and high yields of diol result (34,135).

Methods of using peracetic acid for epoxidation have also been developed. With pre-formed peracid, it is necessary to remove the acidic catalyst by addition of sodium acetate and to operate at temperatures not exceeding 20–25°, usually for 3–4 hr. (34,45,136). On the other hand, polystyrene–sulfonic acid resins, such as Amberlite IR-120 or Dowex 50-X-80, promote formation of the peracid without attacking the epoxide and, with these catalysts, higher temperatures (to 80°) may be safely used and the peracid need not be preformed. The proportion of acetic acid used is reduced to 0.5 mole/mole of olefin (35,46).

Performic acid. Formic acid is itself such a strong acid that the equilibrium with hydrogen peroxide is set up more quickly and an acidic catalyst is not needed. Mixtures of formic acid (90–98%) and hydrogen peroxide (25–30%) at 40° will convert olefins smoothly to hydroxy–formyloxy derivatives (XVI, R = H) usually in 2–4 hr. Since there is no need to isolate or pre-form the peracid, it does not matter that it is less stable than peracetic acid. The formylated glycol is subsequently hydrolyzed by alkali or even with water (steam distillation) (18,34,117,135,136). Epoxidation with performic acid is also possible using smaller proportions of formic acid (0.25–0.5 mole/mole of olefin), though longer reaction periods are then required (96).

Hydroxylation with 30% hydrogen peroxide in formic acid solution at 40° is considered to be the most efficient peracid hydroxyla-

tion procedure (134b), and reaction with 30% hydrogen peroxide in acetic acid solution containing catalytic quantities of sulfuric acid is rated as the second most efficient procedure. More concentrated hydrogen peroxide (90%) offers advantages only for olefins which are resistant to peracid hydroxylation (41).

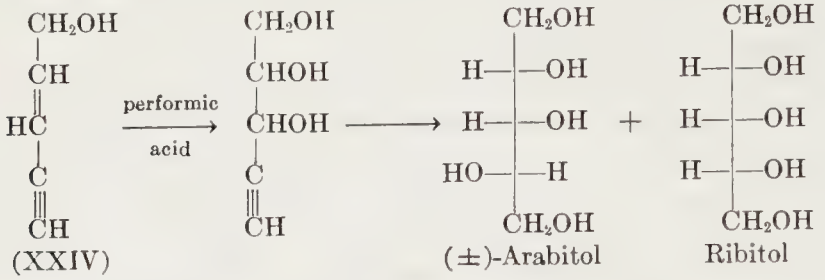
Pertrifluoroacetic acid. This reagent, CF_3CO_3H, prepared from the anhydride and 90% hydrogen peroxide, is very effective for epoxidation and hydroxylation of olefins. It is more reactive than performic acid, hydroxylating not only simple olefins but α,β-unsaturated acids and esters, as well as other olefins carrying negative substituents, in high yields (60–95%); it also has certain advantages in the synthesis of water-soluble glycols (39). Under normal conditions the hydroxy trifluoroacetate (XVI, R = CF_3) is formed and then converted to the glycol by acid-catalyzed methanolysis; in the presence of a suitable buffer, such as sodium carbonate or disodium hydrogen phosphate, epoxides result (70–90%) (40). Pertrifluoroacetic acid is reported to oxidize aromatic hydrocarbons to quinones (94).

D. GENERAL COMMENTS

1. Peracid oxidation of polyethenoid compounds has been studied much less fully. Where the unsaturated centers are widely separated, each acts independently of the other and mono- and diepoxides and/or the diols and tetrols therefrom have been isolated (15,156). Conjugated dienes do not appear to have been studied extensively and unexpected results have been obtained with 1,4-dienes such as occur in several natural fatty acids. Epoxidation of linoleic acid and the further reaction of the diepoxide to acylated glycol appear to proceed normally, but subsequent hydrolysis gives only poor yields of tetrahydroxy acids. The main products are probably oxygen-heterocycles resulting from the 1,4- and 1,5-disposition of hydroxyl groups (80,137; see also 102). Bharucha and Gunstone (10) report that while performic oxidation of 12,13-dihydroxyoleic acid gives poor yields of 9,10,12,13-tetrahydroxystearic acid, satisfactory results follow the use of 12,13-diacetoxyoleic acid. The epoxidation of linoleic acid has also been reported (140).

2. Peracids react with acetylenic centers more slowly than with ethylenic centers, and this important observation (116) has been used in both synthetic (15,117) and degradative (154) studies. The former use is illustrated in Raphael's synthesis of (±)-arabitol and ribitol in

which the *trans*-enyne (XXIV) is hydroxylated at the olefinic center, and the acetylenic group converted to a diol in another way after semihydrogenation (117).



3. Though epoxidation and hydroxylation of monoolefins proceed so satisfactorily in most cases, anomalous results have been described in a few instances. (*a*) Compounds containing the group —CH(OH)·CH:CH— resulting from autoxidation of methyl oleate, when treated with performic acid, gave only 50% of the expected triol along with considerable amounts of acidic fission products (124). Satisfactory oxidations of α- (59) and β-hydroxy olefins (57,135) have been reported. (*b*) Performic acid oxidation of cyclooctene affords not only the expected *trans*-1,2-diol but also the *cis*-1,4-diol (in greater yield) and other nondiol products. Rearrangement accompanies the opening of the epoxide ring and is due to transannular effects (109). Similar results have been observed with the C_9- (109a), C_{10}- (109) and C_{11}-cycloalkenes (109b), and, to a much smaller extent, with the C_6 (109c) and C_7 (109d,109e) compounds. (*c*) 2,4,4-Trimethylpent-1- and -2-enes react normally with perbenzoic acid, but with performic acid and peracetic acids the expected diols are accompanied by unsaturated monohydric alcohols, carbonyl compounds, and dioxane derivatives (22).

VI. Hydrogen Peroxide

The over-all change of olefin to glycol requires addition of two atoms of hydrogen and two atoms of oxygen per molecule of olefin; it is therefore not surprising that efforts have been made to effect this change by means of hydrogen peroxide. These attempts have been made under four different sets of conditions: (*a*) under the influence of ultraviolet light; (*b*) in the presence of an organic acid, especially formic or acetic (Section V-C); (*c*) in the presence of certain metallic oxides, or their derived peracids, capable of acting as hydrogen

peroxide–transfer catalysts, either with or without an organic solvent; and (*d*) in the presence of alkali.

A. HYDROGEN PEROXIDE ADDITION CATALYZED BY ULTRAVIOLET LIGHT

Hydrogen peroxide, exposed to ultraviolet light of about 3000 A. wavelength, is decomposed to free radicals capable of hydroxylating olefinic compounds (85)

$$H_2O_2 \xrightarrow[(3000\ A.)]{h\nu} 2 \cdot OH \xrightarrow{\text{olefin}} R_2C(OH)\cdot C(OH)R_2$$

Optimum results are reported with 10% hydrogen peroxide, but the reaction is slow (5–7 days), does not give high yields, is probably not stereospecific (26), and does not provide a practical method of hydroxylation.

B. HYDROGEN PEROXIDE AND METAL OXIDE CATALYST

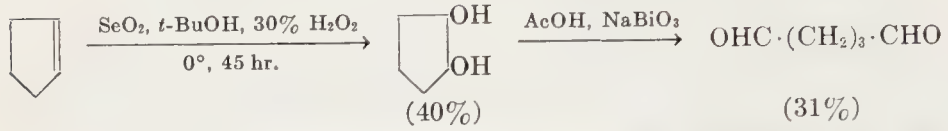
Attempts have been made to replace osmium tetroxide (Section III-D) by other catalysts, and though some oxides are useful and merit fuller investigation, none has yet been so widely used as the well-established osmium tetroxide. The oxides of molybdenum, selenium, and tungsten show some promise but those of chromium, titanium, tantalum, and vanadium are less satisfactory (93).

Treibs recommended the use of hydrogen peroxide and pervanadic acid for the production of epoxides and glycols and considered acetone to be the best solvent (144, cf. 83). Mugdan and Young (93), on the other hand, after a systematic examination of possible catalysts for the oxidation of allyl alcohol in aqueous solution, found that with this catalyst reaction is slower than with osmium tetroxide, large amounts of byproducts are found, and most organic solvents are oxidized.

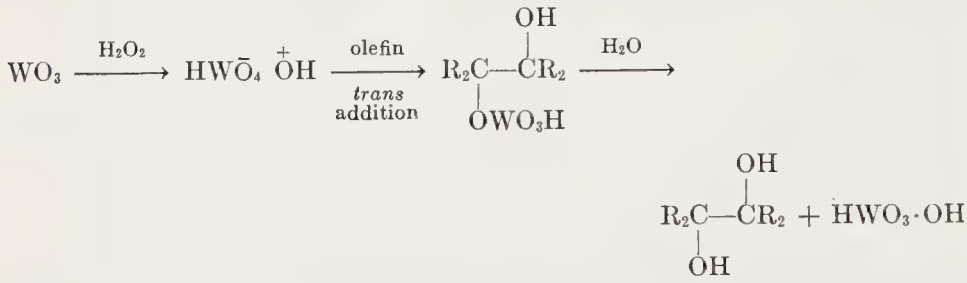
These authors consider, however, that tungsten trioxide may replace osmium tetroxide as a useful catalyst. Though slow at room temperature, reaction proceeds more rapidly at elevated temperatures and in the presence of a trace of sulfuric acid. This oxide has the advantage of being nontoxic, easier to handle than osmium tetroxide, and readily available in large quantities. Several olefins and some olefinic acids and alcohols were satisfactorily oxidized by this means.

Selenium dioxide has found favor with some investigators, being

used in *tert*-butanol, dioxane, acetone, or aqueous solution (93,126, 131). The following reaction, developed as a source of glutardialdehyde, is typical of its use (131).



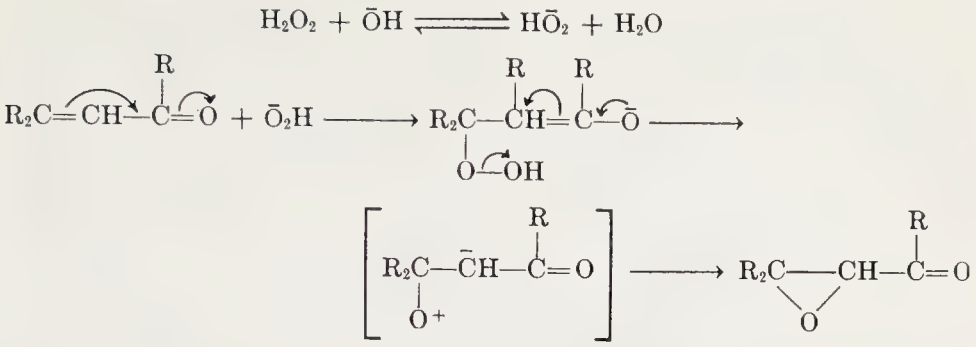
The most interesting feature of the oxidation with hydrogen peroxide catalyzed by these metal oxides is that, although the use of osmium tetroxide leads to *cis* addition, the other oxides promote *trans* addition. This clearly indicates a difference of reaction mechanism, and Mugdan and Young consider that reaction with osmium tetroxide proceeds through the cyclic osmic ester as already reported (Section III-A) but that pertungstic acid oxidation follows the course (93; cf. 76)



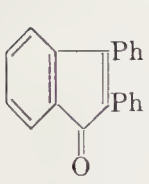
The difference in stereochemistry between these otherwise similar reactions means that in most cases they do not provide alternative routes to the same stereoisomer.

C. HYDROGEN PEROXIDE IN ALKALINE SOLUTION

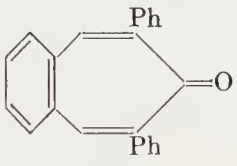
Weitz and Scheffer (149) first showed that alkaline solutions of hydrogen peroxide readily epoxidize α,β-unsaturated ketones in aqueous methanol, ethanol, acetone, or pyridine solution at around room temperature. Saturated ketones, olefins, and α,β-unsaturated acids are unaffected under these conditions but α-diketones suffer fission. Kinetic studies have shown that the rate determining step is attack by the nucleophilic hydroperoxide ion, and the mechanism on page 133 has been suggested (19). Weitz and Scheffer epoxidized in this way several chalcones (Ar·CH:CH·CO·Ph), mesityl oxide, and the compounds XXV–XXVII, of which XXVI and XXVII



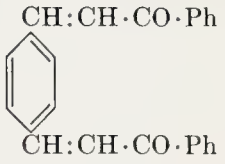
afford diepoxides. The method has also been used for the epoxidation of cholestenone (XXVIII, 104) and of a number of 1,4-naphthaquinones having the general formula XXIX (43,142).



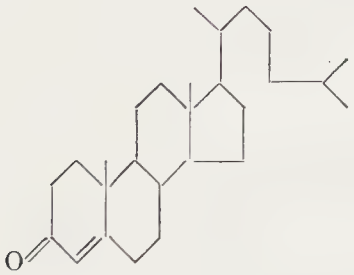
(XXV)



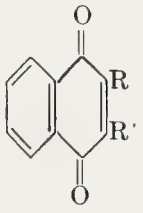
(XXVI)



(XXVII)



(XXVIII)



(XXIX)

The method is of particular value in view of the failure of perbenzoic acid (Section V-B) or of osmium tetroxide in ether (Section III-A) to react with α,β-unsaturated ketones (130).

VII. Experimental

A. PERMANGANATE HYDROXYLATION

1. Oxidation of Oleic Acid in Dilute Aqueous Alkaline Solution (74)

Oleic acid (5 g.) is warmed with a solution of sodium hydroxide (5 g.) in water (500 ml.) until clear, diluted with ice cold water (4

liters), and the whole stirred at 10° during the quick addition of 1% permanganate solution (400 ml.). After 5 min., the liquid is decolorized with sulfur dioxide and acidified with concentrated hydrochloric acid (150 ml.). The precipitated dihydroxystearic acid is filtered under suction, washed with a little petroleum ether (b.p. 60–80°, 50 ml.) to accelerate the subsequent drying, and dried in a vacuum desiccator. The dried product is powdered and extracted with petroleum ether (b.p. 60–80°, 100–150 ml.) to remove saturated acids originally present in the oleic acid, and the insoluble dihydroxystearic acid may finally be crystallized from ethanol. (Yield: almost quantitative; m.p. 132°.)

Full experimental details of the oxidation of acrolein ethyl acetal to (±)-glyceraldehyde ethyl acetal by a similar procedure are available (157).

2. Oxidation of Cyclohexene in Aqueous Ethanol Solution (23)

A solution of potassium permanganate (40 g.) and magnesium sulfate (30 g., anhydrous) in water (800 ml.) is added gradually to a vigorously stirred suspension of cyclohexene (26 g.) in ethanol (600 ml.) during 1.5 hr., the temperature being kept between −15 and −20°. After filtration, the solution is concentrated to a small volume, saturated with salt, and extracted with chloroform to yield the *cis*-diol (12 g., 33%, m.p. 98°).

3. *Oxidation of Bicyclo-[2.2.1]-2-heptene in Aqueous tert-Butanol Solution* (150).

A solution of potassium permanganate (23.4 g.) and sodium hydroxide (5 g.) in water (800 ml.) is cooled to 0° and added quickly with vigorous stirring to a cold (−10°) mixture of *tert*-butanol (1 liter), water (200 ml.), and cracked ice (500 g.) containing bicyclo-[2,2,1]-2-heptene (9.42 g.). Most of the permanganate color is discharged after 3–5 min. and sulfur dioxide is added to insure complete reduction of the permanganate. The precipitated manganese dioxide is filtered through a layer of filter-aid, most of the *tert*-butanol is removed by distillation at atmospheric pressure, and the resultant solution is concentrated to about 250 ml. on the steam bath under reduced pressure. The solution is continuously extracted (48 hr.) with ether, and the ether solution dried over anhydrous sodium sulfate. Evaporation of the solvent gives a white crystalline solid

(5.77 g., 45%). Sublimation (105–110°/18 mm.) gives *exo-cis*-bicyclo-[2,2,1]-heptane-2,3-diol (5.38 g., 40%, m.p. 139.5–140.5°).

Hydroxylation of cyclopentene, cyclohexene, and cycloheptene is effected using this procedure.

B. OSMIUM TETROXIDE HYDROXYLATION

1. Oxidation of Cycloheptene (28)

Cycloheptene (1.92 g.), osmium tetroxide (5.10 g.), and pyridine (3.5 ml.) are mixed in dry ether (100 ml.) at 0°. The mixture sets almost immediately to a mass of brown crystals which is collected after $^1/_2$ hr. and washed with ether (yield 10 g., 98.5%).

The cycloheptene–osmium tetroxide–pyridine adduct (9.5 g.) is shaken with mannitol (10 g.) in 10% aqueous potassium hydroxide solution (100 ml.). It soon dissolves and after 12 hr. is extracted with methylene chloride. After removal of the solvent, the *cis*-diol distills (90–100°/0.1 mm., m.p. 48°) in almost quantitative yield.

2. Oxidation of Carcinogenic Hydrocarbons (25)

A solution (or suspension) of the hydrocarbon (2.5 millimoles) in pure dry benzene (15 ml.) is treated with osmium tetroxide (2.5 millimoles) and pure pyridine (5 millimoles). The color slowly deepens and a dark brown microcrystalline complex is deposited. With 3,4-benzpyrene and 1,2-benzanthracene and its homologs, reaction is complete in 24–48 hr. at room temperature. With less reactive or less soluble hydrocarbons (e.g., anthracene, 1.2.5.6-dibenzanthracene), the reaction requires several days or even weeks at room temperature. With chrysene the reaction is complete in a week at 35°.

The filtered complex is dissolved in methylene chloride and the solution shaken mechanically with 1% potassium hydroxide solution containing 10% of mannitol. The color of the methylene chloride solution fades and the aqueous solution becomes pink with potassium osmate. In some cases (pyrene, 3,4-benzpyrene, 1,2,5,6-dibenzanthracene), the organic layer remains somewhat colored on account of quinone formation. Most of the diols are sparingly soluble and may come out of solution. The solid is filtered off and the remainder of the diol is recovered by evaporation of the methylene chloride

solution. The diols obtained in good yield are recrystallized from benzene, chloroform, or methanol, or purified by chromatographic adsorption on alumina from benzene solution, followed by elution with chloroform. Most of the diols crystallize poorly, some of them separating as gels. However, the diacetates, formed by brief boiling with acetic anhydride containing a little pyridine, are well-crystallized colorless compounds.

3. Oxidation of 4-Chlorocrotonic Acid by Osmium Tetroxide and Barium Chlorate (16b)

4-Chlorocrotonic acid (m.p. 81.5°, 120 g.) is dissolved (partially suspended) in water (1.5 liters) containing osmic acid (0.5 g.) and barium chlorate (60 g.) and allowed to stand at room temperature with frequent shaking at the beginning. The temperature is not permitted to exceed 30°. The acid dissolves completely in about 4–5 hr. and after 20 hr. all the chlorate is used up and the solution becomes black. More chlorate (4 g.) is then added and the solution allowed to stand a further 18 hr. The clear solution is extracted with benzene (2 × 500 ml.) and the aqueous solution concentrated, at a temperature not exceeding 50° and under reduced pressure, to a thin syrup. This is dissolved in absolute alcohol and the solvent again removed under reduced pressure until a thick syrup remains. When this is dissolved in ether (1.5 liters), inorganic matter precipitates and the resulting clear solution is thoroughly dried with sodium sulfate. After removing the solvent, there remains a thick colorless syrup crystallizing soon to a semicrystalline mass which is crystallized from a mixture of ethyl acetate (100 ml.) and chloroform (50 ml.) at −15°. The crystals are filtered and washed with a cold mixture of ether and chloroform (1:3 by volume); the mother liquor is concentrated under reduced pressure and the crystallization repeated. After three successive crystallizations, 109 g. of crystals (m.p. 97–98°) are obtained and a further 7 g. from the residue by repeating the treatment with ether and subsequently crystallizing. The combined crystals (116 g.) are recrystallized from ethyl acetate (250 ml.) to give crystals (105 g., 68%) of m.p. 100°.

When the oxidation is made by gradual addition of barium chlorate and the excess reduced with sulfur dioxide, the desired product is obtained in 78% yield.

4. Oxidation of Allyl Alcohol by Milas' Reagent (88a)

A mixture of 30% hydrogen peroxide (100 ml.) and pure *tert*-butanol (400 ml.) is treated with small portions of anhydrous sodium sulfate, whereupon two layers separate. The alcohol layer containing most of the hydrogen peroxide is dried with anhydrous sodium sulfate and finally with anhydrous calcium sulfate to give a solution containing 6.32% hydrogen peroxide (93.8% recovery) which can be concentrated by vacuum distillation at room temperature without loss of peroxide, provided an all-glass apparatus is used. Only a small decrease in peroxide concentration occurs when the solution is allowed to stand at room temperature over 6 months.

Osmium tetroxide dissolves readily in *tert*-butanol, and the solution is perfectly stable provided no isobutylene is present; otherwise, most of the osmium tetroxide is readily reduced into an insoluble black colloidal oxide which is a very active catalyst for the decomposition of hydrogen peroxides.

To allyl alcohol (6.1 g.) are added hydrogen peroxide reagent (54.6 ml., 6.3%) and osmium tetroxide solution catalyst (1.0 ml.). The reaction mixture warms considerably and has to be cooled. After 3 hr. the peroxide is completely consumed, and the reaction mixture is fractionated to remove the solvent, catalyst, and unreacted allyl alcohol (1.7 g.). The resulting glycol (4.2 g., 60%) is identified as its tribenzoate (m.p. 69°).

C. WOODWARD PROCEDURE

1. Oxidation of dl-anti-trans-4,4a,4b,5,8,8a-Hexahydro-1,8a-dimethyl-2(3H)-phenanthrone (IX) (159; cf. 71)

The unsaturated ketone (IX, 10 g.) is dissolved in glacial acetic acid (analytical reagent, 99.5%, 200 ml.) in a three-neck flask equipped with reflux condenser and thermometer. After addition of silver acetate (16.43 g.), finely powdered iodine (11.69 g.) is added in small portions with vigorous stirring to the reaction mixture over a $^1/_2$ hr. period at room temperature. When all the iodine has been consumed ($^1/_2$ hr.), aqueous glacial acetic acid (19.7 ml. of a solution prepared by diluting 2.0 ml. of water up to 50 ml. with glacial acetic acid) is added. The reaction mixture is then heated at 90–95° for 3 hr. with vigorous stirring. At the end of this time it is cooled, treated with excess sodium chloride, and filtered free from insoluble salts. The

precipitate is thoroughly washed with hot benzene, and the filtrate is evaporated at the pump, taken up in methanol, filtered, neutralized with several milliliters of methanolic potassium hydroxide solution, and then treated with potassium hydroxide (3.1 g.) dissolved in methanol. Hydrolysis proceeds overnight under nitrogen at room temperature. In the morning, the reaction product is carefully neutralized with dilute hydrochloric acid at ice bath temperature. Methanol is removed at the water pump and then under oil pump vacuum to give a crystalline residue (12.2 g.). The crude glycol is dissolved in a large volume of ethyl acetate which is then concentrated until solid separates, at which time the solution is cooled gradually to ice bath temperature. A large mass of fine needles separates from solution. After filtration the *β-cis*-glycol (7.48 g., 65%, m.p. 184–185°) remains, and a second crop (0.71 g., 6%, m.p. 181–183°) is obtained from the mother liquors. After several recrystallizations from ethyl acetate, the *β-cis*-glycol melts at 184.6–185.2°. The mother liquors after removal of two crops of the *β-cis*-glycol give a little of the *α-cis*-glycol (0.28 g., 2.5%, m.p. 156–157°) when seeded with this isomer.

2. Oxidation of Long-Chain Olefinic Compounds (53)

The following method applies to the oxidation of esters. The ethylenic compound (0.01 mole), silver acetate (0.022 mole), and iodine (0.01 mole) in glacial acetic acid (65 ml.) are shaken for 30 min. at room temperature. Wet acetic acid (10 ml. containing 0.2 ml., 0.011 mole, of water) is then added, and the mixture is refluxed for 1 hr. After cooling, the precipitated silver salts are filtered off and washed with a little acetic acid and the solvent removed from the combined filtrate and washings by distillation at 100° under reduced pressure. The residue is diluted with water and extracted with ether and the extract washed with concentrated ammonia and then with water. The solvent is next removed and the product hydrolyzed by boiling it with excess of 3*N* aqueous alcoholic potassium hydroxide for 1 hr.; the mixture is then diluted and acidified with hydrochloric acid. The crude oxidation product is dried in a vacuum desiccator containing concentrated sulfuric acid and potassium hydroxide and crystallized, generally from methanol.

In the oxidation of olefinic acids the (partially) acetylated glycol cannot be washed with ammonia; therefore, after acetic acid has

been removed and water and ether added, the mixture is treated with dilute hydrochloric acid, and the precipitated silver salts are removed and washed with ether. The ether solutions are combined and washed with water, the solvent removed, and the residue hydrolyzed.

With neutral products there is no need to acidify the final hydrolyzate; the product usually separates when the diluted solution is cooled.

D. PRÉVOST REACTION

1. Oxidation of Methyl Oleate (155; see also 153)

A mixture of oven-dried silver benzoate (45.8 g., Ag content 46%), iodine (25.4 g.), and anhydrous benzene (300 ml.) is refluxed on a steam bath for $^1/_2$ hr. with adequate precaution against moisture. Methyl oleate (29.7 g., 96% pure) is then added and the reactants refluxed for 24 hr. Silver iodide is removed by filtration and the filtrate washed successively with water, dilute sodium bicarbonate solution, and again with water. The benzene solution is dried over sodium sulfate and the solvent removed under reduced pressure leaving a reddish oil (43.5 g., 81%).

A portion of this material (10 g.) is stirred intermittently while being heated on the water bath with 10% aqueous sodium hydroxide (50 ml.), and the hot solution is then stirred mechanically during acidification with concentrated hydrochloric acid. An oily layer of dihydroxy acid collects at the surface, and benzoic acid crystallizes in the aqueous layer. When the upper layer has solidified, it is separated and triturated with dilute acid and finally with water. The washings are combined with the aqueous layer which, on heating, yields a little more oily material. This is combined with the original product and dried on a porous plate. The material, dissolved in absolute ethanol and allowed to stand, affords five successive crops of crystals (5.9 g.) which, after two crystallizations from ethyl acetate, melt at 93–94°. Evaporation of the original mother liquor gives a mixture of benzoic and dihydroxystearic acids; these are separated by treatment with Skellysolve B, in which the latter is practically insoluble. The insoluble portion after crystallization from 95% ethanol yields a further 0.5 g. of dihydroxystearic acid (total yield: 5.5 g., 93% on the basis of the benzoxylated product subjected to saponification).

E. PERACID HYDROXYLATION

The following warning is quoted from Swern's excellent review (134b):

"All preparations of and reactions with organic peracids should be conducted behind a safety shield, because a reaction occasionally proceeds with uncontrollable violence. When one olefin of unknown structure or one that contains at least three electron-releasing groups attached to or in close proximity to the ethylenic linkage is epoxidized or hydroxylated for the first time, the reaction should be run on a small scale (preferably 0.1 mole or less) and provision should be made for efficient cooling. Peracid oxidation mixtures should not be distilled unless an analysis has indicated the absence or low concentration of active oxygen. When the peracid content is low, acetic and formic acids can be safely and completely distilled from oxidation reactions at or below room temperature by the use of low pressure. Peracids and other peroxides can be conveniently destroyed by the addition of ferrous sulphate, sodium bisulphate, or other reducing agents."

1. Oxidation of Cyclohexene with Hydrogen Peroxide and Formic Acid (18; cf. 2,123)

Cyclohexene (375 g.), purified by shaking with saturated sodium bisulfite solution, drying (Na_2SO_4), and distilling, is added to formic acid (1500 g., $d = 1.20$) in a 3 liter three-necked flask equipped with stirrer, thermometer, reflux condenser, and dropping funnel. The temperature of the mixture is raised to 45° by passing steam into a water bath surrounding the flask. Hydrogen peroxide (585 g., 100 vol.) is added during 1.5 hr. with stirring (at the interface between the two phases) and cooling, the internal temperature being maintained at 55–60°; the mixture finally becomes homogeneous. Most of the formic acid is removed *in vacuo* (water pump), and the residual crude monoformyl ester is hydrolyzed by steam distillation until 750 ml. of aqueous formic acid distillate is collected. The remaining solvents are evaporated *in vacuo* to give a pale yellow syrup which readily solidifies. One crystallization gives colorless diol (426 g., 82%, m.p. 96–97°, lit. 103.5–104°) sufficiently pure for further reaction.

An alternative procedure for recovering a purer product is described by Roebuck and Adkins (123).

2. Oxidation of Oleic Acid with Hydrogen Peroxide and Formic Acid (135)

Hydrogen peroxide (6.9 g., 25.3% H_2O_2) is added in one portion to a well-stirred solution of oleic acid (14.1 g., 99.5% pure) in formic

acid (42.3 ml.) at 25°. The reaction becomes vigorous after a time lag of about 5 min. and the temperature is maintained at 40° with a cold water bath at the beginning and with a warm water bath toward the end of the reaction. The reaction mixture becomes homogeneous in about 20 min. and there is no further disappearance of peroxide after about 2 hr., approximately the theoretical quantity of hydrogen peroxide being consumed. The product is isolated in one of three ways.

In batches of this size, the formic acid is recovered by distillation under reduced pressure to yield hydroxy–formyloxystearic acids as a residue, or the reaction mixture is poured into cold water and the product extracted with ether. Evaporation of the water-washed ether layer yields hydroxy–formyloxystearic acids as a nonvolatile residue. In larger scale operations (100 g. or more), the reaction mixture is poured into three or more volumes of water, the aqueous layer discarded, and the oil washed several times with water. By all three procedures quantitative yields of hydroxy–formyloxystearic acids are obtained as a low-melting solid.

The product is now heated for 1 hr. with excess 3 *N* aqueous sodium hydroxide, and the pale yellow soap solution poured into excess hot 3 *N* hydrochloric acid with stirring. The oil which forms is allowed to solidify and the aqueous layer discarded. The white solid is remelted with hot water and stirred to remove any residual salts and soluble acids. The white solid (15.6 g., 99%, m.p. 90–92°) obtained on cooling is air dried. A portion (5 g.) of this, finely ground, washed three times by decantation with Skellysolve B (b.p. 63–70°), and filtered, gives dihydroxystearic acid (4.7 g., 93%, m.p. 92°), while another portion (5 g.) crystallized from 95% ethanol (25 ml.) gives a purer product (4.0 g., 79%, m.p. 94°).

Identical results are obtained when 90% formic acid is substituted for the 98% grade, although the reaction mixture never becomes homogeneous.

3. Oxidation of Oleic Acid with Hydrogen Peroxide, Acetic Acid, and Sulfuric Acid (135)

As described above, oleic acid (14.4 g., 98.1% pure) is dissolved in glacial acetic acid (43.2 ml.) containing concentrated sulfuric acid (1.1 g.), heated to 40°, and oxidized at that temperature with hydrogen peroxide (6.75 g., 25.8%). The reaction is not very exothermic

and the reaction time is 6 hr. The hydroxyacetoxystearic acids (quantitative yield) after saponification, acidification, and crystallization afford 9,10-dihydroxystearic acid in slightly lower yield than when hydrogen peroxide and formic acid are used.

4. Oxidation of Pent-2-ene with Pertrifluoroacetic Acid (39)

Trifluoroacetic anhydride (37.2 ml.) is added to a stirred suspension of hydrogen peroxide (6.0 ml., 90%) in methylene chloride (50 ml.) cooled in an ice-bath, and the resulting solution is stirred 10 min. in the cold. It is then transferred while cold to a dropping funnel equipped with a pressure equalizer tube and added dropwise over 30 min. to a solution of pent-2-ene (14.0 g.) and trimethylammonium trifluoroacetate (21.4 g.) in methylene chloride (50 ml.). Throughout the addition, the exothermic reaction causes the solvent to boil vigorously. After addition is complete, the solution is stirred 15 min. at room temperature. The volatile solvents are then removed at reduced pressure and the residue distilled to yield the hydroxytrifluoroacetate (50.5 g., b.p. 38–60°/2 mm.), the triethylammonium trifluoroacetate remaining as distillation residue. The hydroxytrifluoroacetate is refluxed for 2 hr. with 3% methanolic hydrogen chloride (300 ml.), the solvent removed *in vacuo*, and the product distilled to give colorless pentan-2,3-diol (15.1 g., 74%, b.p. 58–59°/0.5 mm.).

Note Added in Proof

The following communications which have appeared since this chapter was written are relevant to the sections indicated.

II-A, II-B: Oxidation of olefins with potassium permanganate in oil/water emulsion. Coleman, J. E., and D. Swern, *J. Am. Oil Chemists' Soc.*, *35*, 675 (1958).

III-A, IV-A: Characterization of the products from oxidation of cholestenone with osmium tetroxide. Eastham, J. F., G. B. Miles, and C. A. Krauth, *J. Am. Chem. Soc.*, *81*, 3114 (1959) .

IV-A: New synthesis of *cis*-cyclohexane-1,2-diol. Brutcher, F. V., Jr., and G. Evans, *J. Org. Chem.*, *23*, 618 (1958).

VI-C: The stereochemistry of base-catalyzed epoxidation. House, H. O., and R. S. Ro, *J. Am. Chem. Soc.*, *80*, 2428 (1958).

VI-C: Stereoselectivity in alkaline epoxidation. Zimmerman,

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THE SELECTIVE DEGRADATION OF PROTEINS

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I. Introduction

In the last 15 years a remarkable transformation has taken place in our understanding of the chemistry of proteins. Whereas in 1944 knowledge of amino acid sequences in proteins was limited to that of a few peptides isolated from partial hydrolyzates (303), it is now possible to write the unique sequence of amino acid residues in many large peptides, including insulin (263) which lies on the borderline between peptides and proteins. Recent investigations have revealed the exact chemical difference between the insulins isolated from various animal species (46,143). Moreover, the degradative methods which proved so successful with insulin, together with new techniques are being applied with striking success to other proteins. Ribonuclease, a protein with 124 amino acid residues, has been intensively investigated, and its chemical structure is almost completely

elucidated (9,217). Studies on lysozyme (mol. wt. 14,700) (310,312) and papain (mol. wt. 20,340) (295) are well advanced. It is significant that the molecular weights of beef insulin of 5732 (269) and of ribonuclease of 13,683 (217) have been precisely determined only through elucidation of their chemical structure. These achievements in structural investigations represent an impressive landmark in biochemical progress.

The traditional method of the organic chemist in determining the structure of complex substances is to degrade them to small compounds which are amenable to purification and identification. From the identity of these fragments, a structure for the parent molecule is deduced and confirmed if possible by synthesis.

For the investigation of the structure of proteins, a whole range of techniques unknown 20 years ago has been developed, including chromatography, countercurrent distribution, and ionophoresis in immobilized media. They have made possible the purification of proteins and the separation of fragments of proteins—peptides, amino acids, and their derivatives. These methods are not only highly efficient; they are adaptable to microgram quantities of material. This has been followed by the development of chemical methods for the identification of amino acids or peptides produced by degradation of a polypeptide (265,276,319).

The possibilities of synthesis presented by a knowledge of amino acid sequences have been rapidly exploited. After having determined the structures of oxytocin (85) and vasopressin (242) by degradative studies, du Vigneaud and his group have elegantly confirmed their structure by synthesis (20,82,84). Other syntheses of oxytocin have been reported (34,35,257,329), and other natural peptides, gramicidin-S (279) and hypertensin (255,278), have also been synthesized.

Protein chemists have built even more interesting molecules. The first hormone to be specifically tailored, oxypressin (171), combining something of the chemical structure of both oxytocin and vasopressin and possessing the biological properties of both parent molecules, is a masterly demonstration of the vast practical and theoretical possibilities opened up by our increased knowledge of peptide chemistry.

For the larger proteins, our methods at the present time are not ideally suited for an attack on the complete amino acid arrangement, and efforts are being made to reduce the problem to an understanding of the "active center" of the molecule. For example, papain, con-

taining 178 residues, can be degraded enzymically until it is only one-third its original size and still retains full enzymic activity on a molar basis (148). Moreover, an —SH group is essential for this activity and acts as a marker for the active site. The enzymic properties of trypsin and chymotrypsin are acquired by the splitting of only one peptide bond in the inactive parent molecules (223,224,256).

From our knowledge of the end groups of a large number of proteins, it appears that many simple proteins contain chains of over 100 amino acids. The chains of insulin with 21 and 30 amino acids are exceptional, although chymotrypsin has been shown by Meedom (213) to consist of three chains, two of which contained 13 and 50 residues, respectively. With chains of this size, where a number of amino acids occur only once in the molecule, it is possible to deduce the structure from the identification of small peptides obtained by relatively nonspecific types of degradation, e.g., partial acid hydrolysis.

In longer chains, very few, if any, residues will occur singly, and many may occur five or more times. In these cases, more selective methods of hydrolysis are required, and it is necessary to isolate longer peptides to allow deduction of the amino acid sequence. These are often more difficult to fractionate, and each isolated peptide must be quantitatively analyzed and have its amino acid sequence determined.

It would simplify the problem considerably if methods were available for specifically cleaving a limited number of peptide bonds, e.g., those involving only one particular amino acid or related amino acids. The selective degradation of protein molecules is the ideal of the peptide chemist, but at the present time is far from being a reality.

The requirements of an ideal reagent would be: (*1*) It should react under conditions where other amino acids are not destroyed. There should, preferably, be no racemization. (*2*) The cleavage should proceed in good yield at each of the selected residues. (*3*) The fragments produced by the cleavage must be capable of fractionation and purification.

The nearest approach to the ideal selective degradation is furnished by the natural enzymes. Of these, trypsin has shown the most exacting specificity. Other proteinases have shown divergencies from the specificity expected through a study of their behavior with synthetic substrates. Trypsin, however, has so far been shown to attack only those bonds involving the carboxyl group of lysyl or arginyl residues which bear a positively charged side chain. Although no cases have

been established of other types of bonds being broken by trypsin, there have been several instances of bonds involving these positively charged residues being resistant to hydrolysis.

It is also possible for enzymes to catalyze the formation of bonds which were not originally present in the molecule (131,332). Although the increased use of proteolytic enzymes in structural investigations has not revealed any detectable synthesis of new bonds, it has been necessary to demonstrate that this does not happen by the use of at least two enzymes of different specificity, usually trypsin and chymotrypsin, which are unlikely to synthesize the same bonds.

The organic chemist has so far made a comparatively poor showing in devising methods for selective degradation. However, the increasing knowledge of amino acid sequences and the standardization of end-group techniques for detecting peptide bond splitting make investigation of possible new methods more attractive. The availability of insulin with authentic analytical data (222) and of other proteins in relatively pure condition will stimulate further efforts on the problem of selective degradation.

Unfortunately, it is usual for chemists working on new types of reactions on peptides to use simple model compounds, including the di- or tripeptides, that are commercially available. This is due partly to the increased difficulties in following the course of reactions with more complex peptides or proteins, which, in any case, may not be readily available. Often they have been isolated in comparatively small quantities and their structures determined on relatively minute amounts of material. Where possible, it would be desirable to have available, commercially, peptide samples of known structure and purity comparable to the samples of insulin organized by the Commission of Proteins of the International Union of Pure and Applied Chemistry.

This article summarizes the chemical and enzymic methods for selective degradation of peptide chains. It has not been possible to include references to all the relevant papers and frequently preference has been given to review articles or summarizing papers.

II. Nomenclature and Types of Peptide Chains

Peptide chains consist of a number of amino acids linked together by means of peptide bonds between the α-amino and α-carboxyl groups. They may be open, cyclic, or branched. In general, open

peptide chains have a free α-amino group at one end and a free α-carboxyl group at the other, which may be detected by various end-group methods (114,276,319). In a branched chain, either group may be absent depending on the type of branching. The majority of proteins are of the open-chain type (264,276).

The suggestions of Sanger and Tuppy (270) regarding nomenclature have been universally adopted. Residues bearing a free α-amino group are referred to as *N*-terminal, and those bearing a free α-carboxyl as *C*-terminal. To facilitate the writing of amino acid sequences, abbreviations for amino acid residues are used, consisting, in general, of the first three letters (41). The *N*- and *C*-terminal residues may be differentiated from residues within the chain by the substituents H- and -OH, respectively.

Where the order of residues in a peptide is known, the symbols are joined by a period. Thus H.Gly.Ala.OH signifies the dipeptide glycylalanine. H.Gly.(Ala, Leu).OH indicates a peptide in which glycine has been identified as the *N*-terminal residue but in which the order of the alanine and leucine is unknown (265). Unless otherwise noted, all optically active amino acids are to be regarded as being of the natural L-configuration.

It should be realized that failure to discover *N*- or *C*-terminal amino acid residues may be due to the inadequacy of available techniques to detect them. Moreover, there have been several cases where an open-chain polypeptide has had either the *N*-terminal residue, the *C*-terminal residue, or both residues masked by covalently bound acyl or amide groups. Thus, oxytocin (85,325), vasopressin (242), and α-melanocyte-stimulating hormone (139) have *C*-terminal amide groups. In tobacco mosaic virus (38), α-melanocyte-stimulating hormone (139), and polymyxin B_1 (144) the terminal amino group is masked by a substituent.

Cyclic molecules do not occur as frequently in proteins as was thought several years ago. With the introduction of more refined methods of terminal group analysis, new end groups have been detected. Thus, chymotrypsinogen has an *N*-terminal half-cystine residue (29,213), and ovalbumin a *C*-terminal proline residue (229). Bovine growth hormone has two *N*-terminal residues but only one *C*-terminal residue, suggesting a branched structure, whereas growth hormones from monkeys or humans have only one *N*-terminal and one *C*-terminal residue (196). At the present time collagen (36,126)

and keratin (40,316,343) are the only proteins where end groups in stoichiometrically significant amounts have not been detected. However, cyclic peptides are known, such as gramicidin S (64), tyrocidin A (231) or B (179), and phalloidin (214,341).

The ways in which peptide chains may be crosslinked has been a matter for discussion for many years (53,304). The disulfide bond of cystine is the most common covalent crosslinkage. More recently, phosphate groups have also been detected in crosslinkages (236). Both diester and pyrophosphate bonds have been demonstrated, and, as in the case of the sulfur bridges, the phosphorus can link two different peptide chains (interchain bonds) or may serve to connect two sites of a single chain in a cyclic loop (intrachain bonds).

Other types of crosslinking are possible but have yet to be experimentally proved in proteins. Thus, evidence has been presented (169,182) for peptide linkages involving amino groups and the ω-carboxyl groups of isoglutamine or isoasparagine residues in insulin, gliadin, and chymotrypsin, based on the Hofmann degradation of amide groups (Fig. 1). This could not be confirmed (268) in the case

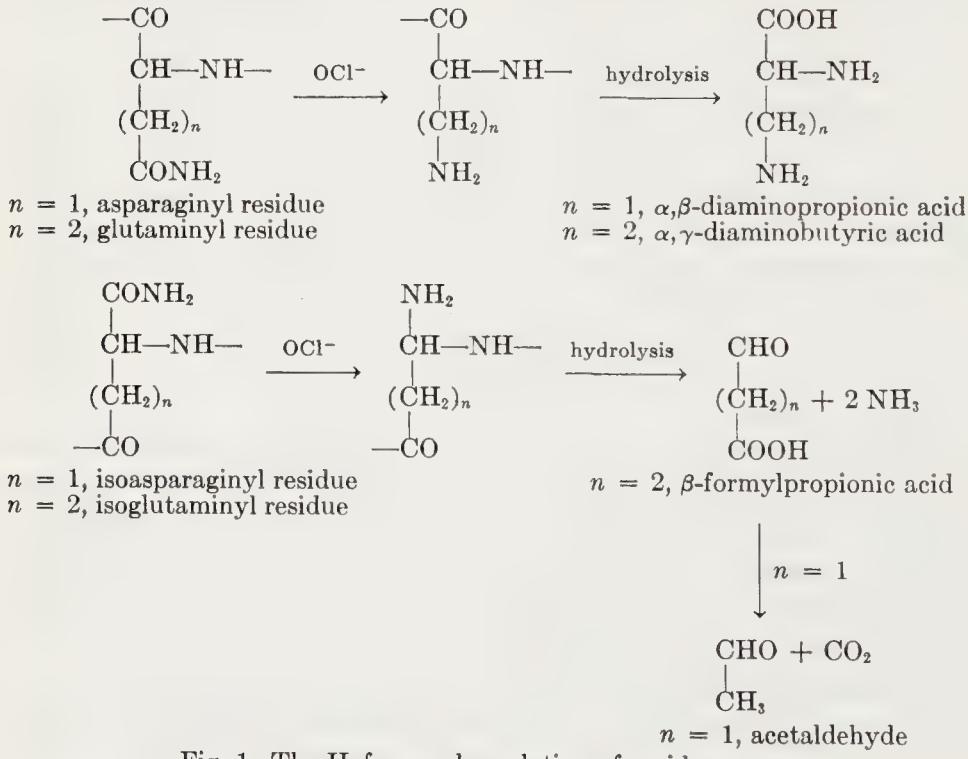
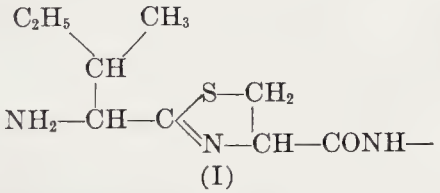


Fig. 1. The Hofmann degradation of amide groups.

of insulin. However, with the peptide from *B. subtilis*, the Hofmann degradation gave crystalline derivatives of β-formylpropionic acid (48), consistent with a γ-linked polyglutamic acid, and no detectable quantities of α,γ-diamino butyric acid. Since synthetic poly-L-glutamine readily gave this latter compound (47), it was concluded that the natural poly-D-peptide probably contained only γ-glutamyl bonds, and this has been substantiated in a recent study (59).

The occurrence of unusual types of bonds in peptides is an indication that biological mechanisms exist for their synthesis and they could be present in proteins. Analytical difficulties with proteins make it difficult to detect a single residue—e.g., an ϵ-amino group of lysine—involved in an unusual type of linkage. However, in smaller peptides such as bacitracin A and polymyxin B_1 there is evidence to show that unusual bonds are present. Thus, in bacitracin A, an ϵ-amino group of lysine and a β-carboxyl group of aspartic acid are involved in a peptide bond to form a cyclic loop in the molecule (145,204). There is also evidence (205,336) for a decreased reactivity of an isoleucyl α-amino group and an —SH group on adjacent residues, which is probably due to formation of a thiazoline ring (I). In



(I)

polymyxin B_1, branching occurs through the amino groups of α,γ-diaminobutyric acid (144).

III. Separation of Component Chains

The breaking of the crosslinks between the component peptide chains of a protein is probably the most generally selective of all degradative approaches and is an essential preliminary to the separation of the chains.

A. OXIDATION OF DISULFIDE BONDS

Oxidation of —S—S— linkages to —SO_3H groups is readily accomplished with many oxidizing agents, but fewer side reactions occur with peracids, such as performic acid or peracetic acid.

Performic acid, which is readily made from formic acid and hydro-

gen peroxide (321), was used by Sanger (262) in preparing the component chains of insulin. All the amino acid residues of the glycyl chain of oxidized insulin were released in stoichiometric amounts (149) by digestion with leucine aminopeptidase, showing that they are all of the L-configuration and that no racemization occurs during the oxidation. Performic acid has now been used with many other peptides and proteins, and no evidence of peptide bond fission has been reported. The only amino acids which are modified by the reagent, apart from cystine and cysteine, are methionine, which is converted to the sulfone (150) in 90% yield, and tryptophan, which takes up three atoms of oxygen (321). Proteins containing tryptophan (e.g., lysozyme) darken on oxidation with performic acid, but no breakdown of peptide bonds takes place in lysozyme (318) or papain (178). Tyrosine may be lost under certain conditions of oxidation (150) and in the presence of chloride ions may be partially converted to 3-chlorotyrosine and less frequently to 3,5-dichlorotyrosine (314). With special precautions to avoid concentration of hydrogen peroxide and chloride ions, this modification of tyrosine may be prevented (221,314).

One aspect of the use of performic acid which requires investigation is the nonquantitative yield (156,178,184,315), 90 ± 2% (275), of cysteic acid obtained from cysteine, cystine, and proteins of established cystine content. There is no information as to the byproduct of the reaction. In the case of insulin, for example, where two interchain cystine bonds must be oxidized to release the chains, the data suggest a maximum yield of 0.9 × 0.9, or 81% yield, of the separated chains with sulfonic acid groups. If the interchain bond is also considered, the maximum yield of the separated fully oxidized chains should be still less. Although near quantitative yields of the separated chains have been claimed (239), the data cannot be considered accurate (315).

In the commercial treatment of wool (5), peracetic acid is preferred to performic acid since it is water stable and does not dissolve any wool. Performic acid is decomposed by water and takes considerable and variable amounts of wool into solution (33). The specificity of peracetic acid is similar to that of performic, but precise information on the quantitative aspects of the oxidation is lacking.

In structural investigations of proteins, preliminary oxidation not only releases the component chains but also unfolds them, thereby

making them more susceptible to complete enzyme digestion. For example, the tryptic digestion of native ribonuclease is only 10% of that with oxidized ribonuclease (150), in which the majority of the susceptible bonds were 85–100% split.

An alternative method of oxidation of cystine crosslinkages is with bromine or bromine water. Sulfonic acid groups are again produced, and with cystine the yield of cysteic acid is quantitative. However, attempts to oxidize the cystine of insulin or papain with bromine did not give good recoveries unless the proteins were first partially hydrolyzed (315). Denaturation may be of value in increasing the extent of reaction, as is the case with reduction methods. Oxytocin, a smaller peptide, gave good yields of cysteic acid on oxidation with bromine water, but specific fission of a tyrosylisoleucyl bond also occurred (see Section IV-B-2).

B. REDUCTION OF DISULFIDE BONDS

The reduction of disulfide bonds is more specific than oxidation in that tryptophan and methionine are not modified by the reagents. Simple thiol compounds such as thioglycollic acid, mercaptoethanol, or cysteine are normally used and the equilibrium reactions

$$R_1\text{—S—S—}R_2 + R_3SH \rightleftharpoons R_1SH + R_2\text{—S—S—}R_3$$

$$R_2\text{—S—S—}R_3 + R_3SH \rightleftharpoons R_2SH + R_3\text{—S—S—}R_3$$

are driven in the desired direction by excess reagent.

In contrast to oxidation with peracids, however, there is a marked difference in the reactivity of the cystine bonds (109,200,237), and usually complete reduction is only achieved under special conditions. Because of the ease of oxidation of —SH groups, particularly at alkaline pH values, the reduced protein is usually stabilized by coupling with iodoacetic acid, iodoacetamide, or some other thiol-blocking reagent.

$$RSH + ICH_2CONH_2 + OH^- \longrightarrow RSCH_2CONH_2 + H_2O + I^-$$

To obtain complete reduction, it is usually necessary to use both a thiol and a reagent causing breakage of hydrogen bonds, thereby unfolding the peptide chains. Thus, insulin has only been completely reduced with thioglycolate in the presence of 8*M* urea (201), and paper electrophoresis in 8*M* urea will allow separation of the two polypeptide chains. In the absence of urea a maximum of two-thirds

of the cystine was reduced. The extent of reduction of the disulfide bonds of ribonuclease (281) is also determined by urea concentration. Of the 17 cystine bonds in both human and bovine albumins (mol. wt. 65,000), only one was reduced by thioglycolate at pH 7 in the absence of detergent (208) or guanidine (170). However, with a thiol in 0.2*M* sodium decyl sulfate solution at pH 7 for 1 hr. at room temperature, all the bonds were reduced.

Thiol-disulfide interchange reactions between neighboring protein molecules have been cited (157) as the explanation of the gelation of proteins in the presence of urea. By breaking hydrogen bonds and unfolding the molecules, urea enables small amounts of —SH groups to break intramolecular cystine links and re-form intermolecular cystine links so that a more crosslinked structure is formed. A similar type of reaction is responsible for the interchange that occurs in cystine peptides during hydrolysis of insulin (259) in either acid or alkaline solutions. Many more cystine peptides were isolated than would be expected from a unique structure. Different mechanisms operate, and inhibitors, —SH-blocking reagents at alkaline pH values and thiols at acid pH values, have been found to prevent rearrangement.

A wide variety of acid or alkaline reducing agents can be used for the reduction of cystine in proteins, but thiols are usually preferred because of the gentle reaction conditions and absence of side reactions. However, sulfite or bisulfite have also been used extensively, and these reagents with cystine yield one mole of cystine and one mole of cysteine-*S*-sulfonate (61,207). Recent studies by Cecil and McPhee (52,211) of the kinetics of the reaction of sulfite with simple disulfides have shown that, whereas above pH 9 the reaction is a simple bimolecular one

$$R—S—S—R + SO_3^{2-} \rightleftharpoons R—S—SO_3^- + RS^-$$

$$RS^- + H^+ \rightleftharpoons RSH$$

below pH 9 it is more complex. The reactive ingredient has been shown to be SO_3^{2-}, and not HSO_3^-, and the electrochemical environment has a marked effect on the rate of reaction. Thus, negatively charged —COO^- groups near to —S—S— bonds are associated with decreased rate constants, and positively charged groups with increased reaction rates.

The reaction of SO_3^{2-} with insulin is an example of differences in

reactivity (51). Thus, at pH 2.8–5.5, a slow reaction causes splitting of only one disulfide bond; at pH 6.2–7.2, the reaction is much more rapid, two bonds being split; while above pH 7.2, the reaction is reversible owing to ionization of the thiol groups; and only 0.4 equivalent of disulfide bonds is split at pH 9. The reaction can be driven to completion at pH 6.5–7.0 in 8*M* urea or 3*M* guanidinium chloride, or at pH 7.0–9.0 in the presence of phenylmercuric hydroxide.

For degradative work on proteins, it is preferable to split the —S—S— bonds quantitatively prior to separating the component chains.

An alternative method of driving the reaction to completion has recently been investigated (16,302). Treatment with sulfite under conditions where the thiol is reoxidized to disulfide, which then reacts with another mole of sulfite, enables complete conversion to —S—SO_3^- groups (61). In contrast to cysteine, the —S—SO_3^- group is stable to air, and this derivative has been recommended (302) as a means of stabilizing cysteine compounds for paper chromatography.

Insulin treated with SO_3^{2-} and an oxidizing agent gives high yields of its component peptide chains (16,302). Moreover, the —S—SO_3^- group is readily converted to other interesting groups, such as —S—CN, and may be decomposed to the thiol again (302).

Compounds with —SCN groups are readily formed from disulfides by reaction with cyanide solutions

$$R—S—S—R + CN^- \longrightarrow RS^- + RSCN$$

$$\text{CH—CH}_2\text{—S—S—CH}_2\text{—CH} + CN^- \longrightarrow$$

$$\text{CH—CH}_2\text{—S}^- + NCS—CH_2—CH$$

$$\text{CH—CH}_2\text{—SCN} + OH^- \longrightarrow \underset{\text{(II)}}{\text{C=CH}_2} + SCN^- + H_2O$$

$$\text{CH—CH}_2\text{—S}^- + \underset{\text{(II)}}{\text{C=CH}_2} + H_2O \longrightarrow$$

$$\text{CH—CH}_2\text{—S—CH}_2\text{—CH} + OH$$

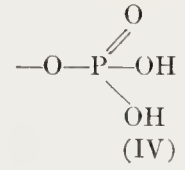
(III)

However, the thiocyanate group in proteins is readily lost by a β-elimination reaction (308) to give thiocyanate ion and an amino acrylic acid residue (II). Recombination between the amino acrylic acid residue and a cysteine residue will give a lanthionine residue (III), which is readily formed from wool or other proteins treated with cyanide or alkaline solutions (315). This method is consequently of no value for separating the component peptide chains of a protein, but may have application in selective cleavage of the peptide chain (see Section IV-B).

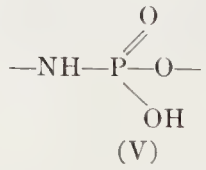
C. CLEAVAGE OF PHOSPHATE CROSSLINKS

All phosphate crosslinkages are unstable in the presence of alkali, e.g., 0.25*N* NaOH at 25° (236). Selective cleavage of the various types has been achieved by the use of phosphatases, which in simple substrates exhibit a specificity for different kinds of phosphate linkage (236). Once transformed into monoesters, the phosphate involved in crosslinkages may be released in the inorganic form with the aid of a variety of phosphomonoesterases from plant or mammalian tissue.

The different enzymes give both a qualitative and quantitative picture of the linkages in a protein. Thus, α-casein contains 40% of its phosphorus as monoesters (IV), 40% as amidoesters (V), and 20% as pyrophosphate (VI). In β-casein, all the phosphorus is present in the form of diester linkages (VII), between peptide chains. A breakage of the crosslinkages leads to splitting of both α- and β-casein into smaller fragments.



(IV)



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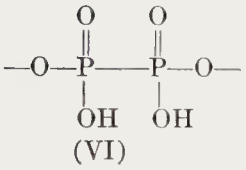

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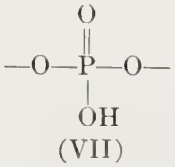


(VII)

In pepsin, with only one phosphorus atom per molecule, a diester of type VII is present. This must form an intrachain bond, since it is known that pepsin consists of a single polypeptide chain (146,328).

D. OTHER TYPES OF CROSSLINKAGES

For most proteins, sufficient cystine is indicated by amino acid analyses to allow for disulfide bridges between the known number of open peptide chains. However, horse hemoglobin, with six *N*-terminal valyl residues (159,244), has no cystine (160); and avidin, with three *N*-terminal alanyl residues (115), has only one cystine residue (322). Obviously, either branched structures or other types of crosslinking must be present.

It is now well established that native proteins exhibit a stability to many types of reagents which can only be explained on the basis of strong interactions between various groups in the peptide chain or between the peptide chains of various molecules. The contribution of hydrogen bonds to the stability may be considerable (186), and the separation of two nonpolar side chains in aqueous solution requires more energy than would be calculated from van der Waals attraction. This is attributed to the energy required to separate those water molecules held together by hydrogen bonds which will be broken during establishment of equilibrium between the nonpolar groups and water (173,174). These interactions between small molecules and proteins (181) and between protein molecules (334) have been reviewed recently and may be illustrated briefly by the behavior of insulin in solution.

The chemical unit of insulin has a molecular weight of 5732 (260, 269). However, the monomer is only obtained in solution under special conditions, such as in aqueous guanidine hydrochloride (183), in organic solvents (134,250), or at low concentrations in solutions of low ionic strength when the protein has a high net charge (116,135). As the ionic strength is raised and the net charge is decreased, there is a progressive association of monomers, resulting, eventually, in the formation of an insoluble isoelectric precipitate (pH 5.3–6.1). Moreover, heating insulin in acid solution results in the formation of a precipitate which consists of an association of insulin fibrils. These fibrils are formed even in 6*M* urea (334). It seems likely that the interactions of nonpolar residues are mainly responsible for the stability of the fibrils, which can, however, be regenerated to crystallizable insulin by treatment with alkali (333).

Horse hemoglobin, which contains no cystine, is particularly interesting in that it has been shown (129) by the surface balance technique to have a molecular weight of 12,000. Hydrodynamic methods

give a molecular weight of about 68,000, which can be reduced to about half of this value in urea solutions (344). If the association is due to secondary valency forces, it might be expected that only "fierce" reagents (306) such as urea, phenols, guanidinium compounds, detergents, and organic solvents like dimethylformamide would be capable of acting as dissociating agents, and, in addition, extreme pH values may be required. Further data on this question are necessary.

E. METHODS OF SEPARATION

Once the component peptide chains in a protein are released, it may be possible to devise ways to separate them in a pure condition. This may be more difficult with long peptide chains (305) than with short peptides for which a variety of separation techniques are readily applicable, such as paper (319) or column chromatography (218,276), countercurrent distribution (330), or ionophoresis (10,265). In the case of oxidized insulin, where there is a marked difference in the acidities of the two chains due to their amino acid content, separation can be achieved either by salt fractionation (262), ionophoresis (143,262), partition chromatography (6), or countercurrent distribution (189,239). Oxidized chymotrypsin, with three component chains, has also given three fractions with distinctive properties (213), enabling them to be separated by a combination of precipitation at pH 6 and ion exchange chromatography of the soluble material.

IV. Reagents Splitting Internal Peptide Bonds

Reagents, such as concentrated acids, which attack all peptide bonds in a random manner are of limited value for long chain peptides, since the complexity of the hydrolyzate increases enormously as the length of the chain increases. General aspects of hydrolysis of peptides have been discussed in detail elsewhere (265), and it is only necessary to emphasize here that there is an optimum time of reaction for hydrolysis of a peptide or protein before attempting fractionation of the mixture of peptides and amino acids produced.

A. ENZYMES

With enzymes, the optimum time will be determined by the purity of the enzyme and the relative rate of splitting of various bonds. The rate of splitting of particular bonds by other contaminant proteinases will, in general, be much slower, because of their low con-

centration, than the rate of splitting of bonds conforming to the specificity requirements of the major component. By stopping the digestion when the rate of increase in amino groups becomes steady, the nonspecific cleavages will be minimal. Bacterial contamination should be avoided, particularly during long digestions, by the addition of preservatives; e.g., 1 part in 10,000 of merthiolate gives good protection. Methods commonly used to follow the course of proteolysis have been described in detail (70). In addition, the pH-stat (163) and end-group analysis (114) may be useful. The latter method has the advantage of following the splitting of individual bonds, whereas other methods estimate the total number of bonds split.

To date, only two endopeptidases have been shown to be sufficiently specific in their action for reasonable predictions to be made, on the basis of amino acid composition of the substrate, of the number of bonds likely to be split. Both these enzymes, trypsin and chymotrypsin, are fairly pure as obtained commercially, but a small amount of cross contamination is possible since both are isolated from pancreatic extracts. Chymotrypsin is inhibited by indole or β-phenyl propionate, and, since chymotrypsin is more readily inactivated by acid (248) or urea (137; but cf. 226), a preliminary treatment of trypsin with one of these reagents may be useful before digestion experiments. Trypsin may be specifically inhibited by a number of low molecular weight proteins, and these have been the subject of a recent review (128). It is only when the specificity of the impurity is high that suspicions are likely to be raised regarding the agent responsible for unexpected cleavages.

Digestion of native proteins is, in general, less extensive than with the denatured or unfolded molecule (128). In many cases this more specific cleavage results in larger fragments. The increased digestibility of ribonuclease after oxidation has been mentioned earlier.

Enzymes are more stable at low temperatures, and reaction mixtures are often incubated at 25°. However, like most chemical reactions, an increase in temperature causes a marked rise in reaction rates, and digestions at 37–40° are often employed to speed up the hydrolysis of bonds not rapidly split by the enzyme. Higher enzyme/substrate ratios will also increase the rate of proteolysis.

In this section the specificity of proteolytic enzymes will be discussed in relation to the selective degradation of peptides and proteins of known amino acid sequence. It must be remembered, of course,

that the sequence of amino acid residues in a chain does not entirely specify the spatial relationships. With coiling of the chain into a folded configuration, such as the α-helix, the side chains of successive residues emerge from the core of the helix at regular intervals and are also rotated with respect to each other through an angle of approximately 100° about the helical axis. Freedom of rotation of side chains allows considerable variation in their position, and they may be as close to another side chain or peptide bond several residues further along the main chain as they are to their adjacent amino acid residue or peptide bond. Moreover, interactions may occur between side chains and peptide bonds geometrically adjacent but far removed in sequence or even belonging to another peptide chain in the molecule. Thus, a knowledge of the stereochemistry may be as important to this inquiry as the amino acid arrangement.

It is also important to know whether aspartyl or glutamyl, rather than asparaginyl or glutaminyl, residues are present.

1. Trypsin

The properties of trypsin have been discussed fully elsewhere (128,227,293) and will be briefly summarized.

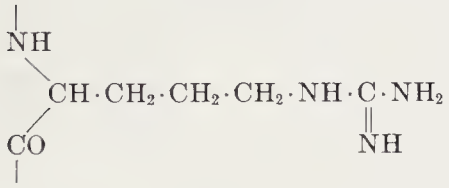
The active enzyme (mol. wt. 23,000–24,000), (128) is formed from inactive trypsinogen by the action of trypsin (227) or enterokinase (345), with the splitting of a -Lys.Ileu- bond and the release of a peptide H.Val.$(Asp)_4$.Lys.OH (69,76) from the *N*-terminal end of the single polypeptide chain. Thus, valine and isoleucine are the *N*-terminal residues of trypsinogen and trypsin, respectively (75). Lysine is possible the *C*-terminal residue in both (104,225; but cf. 223).

The pH range for trypsin activity is 7–9, with optimum activity for some synthetic substrates at pH 7.8 (128). The enzyme readily autolyzes in this pH range and has a maximum stability at pH 2.3 at 30° (227). Although more stable in the presence of substrate, it has been found that a trypsin solution at pH 7.9 and 26° loses 50% of its activity within 30 min. in the absence of Ca^{2+}, and is completely inactivated in 6 hr. (125). The inactivation of the enzyme is greatly retarded by Ca^{2+} (31,77,125). Thus, under the conditions given above but in the presence of 0.01*M* Ca^{2+}, only 5% inactivation occurred in 8 hr.

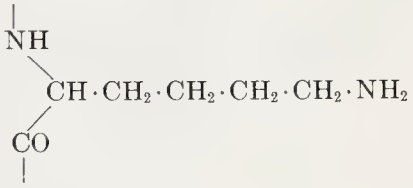
Acylation of trypsin also stabilizes the enzyme (245). Since the

trypsin molecule contains thirteen lysine residues and only two arginine residues (128) which form potentially hydrolyzable bonds during self-digestion, acylation of the ϵ-amino groups of lysine residues makes bonds involving these residues resistant to hydrolysis. This reduces the number of susceptible bonds and hence increases the stability.

Experiments with synthetic substrates (27) have shown that trypsin will attack peptide bonds to which an L-arginine (VIII) or L-lysine (IX) residue has contributed the carboxyl group. Typical substrates are benzoyllysineamide or benzoylarginineamide. If the ϵ-amino group of the lysine residue is covered by a substituent, the sensitivity to trypsin disappears.

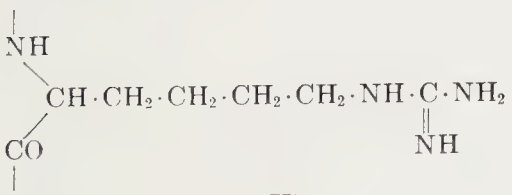


(VIII)

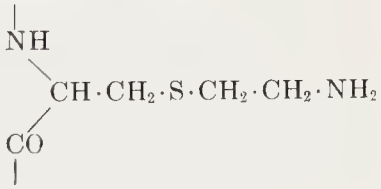


(IX)

Recently, it has been shown that conversion of the ϵ-amino group of lysine in α-lactalbumin into guanidyl groups, corresponding to homoarginyl (X) residues, also renders the previously susceptible peptide bonds completely resistant (335). On the other hand, conversion of cystine residues into *S*-(β-aminoethyl)-cysteine residues (XI), by reduction with thioglycolate followed by coupling with 2-bromoethylamine, produces a side chain closely resembling that of lysine. Poly-*S*-(β-aminoethyl)cysteine is a substrate for trypsin (203) in the same way as polylysine. The introduction of these residues into polypeptide chains in place of half-cystine residues will increase the digestibility by trypsin. This type of conversion may be useful in amino acid sequence investigations of proteins (203,320). Histidine or ornithine cannot replace arginine, lysine, or *S*-(β-aminoethyl)-cysteine residues.



(X)



(XI)

TABLE I
Action of Trypsin on Various Proteins

Peptide or protein	Bonds readily split		Bonds partially split	Resistant bonds involving Lys or Arg
Oxidized insulin, B chain	Arg. Gly (22 23)	Lys. Ala (29 30)		
Glucagon	Arg. Arg (17 18)	Lys. Tyr (12 13)	Arg. Ala (18 19)	
Vasopressin	Arg. Gly. NH_2[a] (8 9)	Lys. Gly. NH_2[b] (8 9)		
Oxidized ribonuclease	Arg. Ser (10 11)	Lys. Phe (7 8)	Arg. $CySO_3H$ (39 40)	H. Lys. Glu (1 2)
	Arg. Asp(NH_2) (33 34)	Lys. Ser (31 32)		Lys. Pro (41 42)
	Arg. Glu (85 86)	Lys. Asp (37 38)		
		Lys. Asp(NH_2) (61 62 also 66 67)		
		Lys. Tyr (91 92)		
		Lys. Thr (98 99)		
		Lys. His (104 105)		
Corticotropin	Arg. Try (8 9)	Lys. Lys (15 16)	Arg. Arg (17 18)	Arg. Pro[c] (18 19)
		Lys. Val (21 22)	Lys. Arg (16 17)	Lys. Pro (11 12)
Melanocyte-stimulating hormone				
α-MSH	Arg. Try (8 9)			Lys. Pro (11 12)
β-MSH	Arg. Try (11 12)	Lys. Met (6 7)		Lys. Asp. OH (17 18)
Hypertensin	Arg. Val (2 3)			

[a] Beef.
[b] Hog.
[c] But see text.

A positively charged free amino group in the α-position has prevented hydrolysis in certain cases but not in others (see below and pages 170 and 172). There is evidence from studies on synthetic substrates that either a positively charged α-amino group or a negative charge in the vicinity of the susceptible bond retards tryptic hydrolysis.

The digestion of polylysine by trypsin (331) illustrates the effect of α-amino and carboxyl groups. Dilysine is relatively unreactive to trypsin. Activity increases markedly with the number of lysine residues from tri- to pentalysine. Higher polymers are readily attacked at bonds near the end to give dilysine and trilysine, but the end peptide bonds in all cases are relatively resistant and only small amounts of free lysine are formed. More recently, Waley and Watson (332) have withdrawn their earlier claim that dilysine is completely unreactive to trypsin. At higher enzyme concentrations, reaction does take place, but the formation of lysine and trilysine is by a transpeptidation (cf. 192) mechanism:

$$2\,\text{H.Lys.OH} \longrightarrow \text{H.Lys.OH} + \text{H.Lys.Lys.Lys.OH}$$

Salmine, which contains repeating arginylarginyl sequences, has also been found (216) to give free arginine during tryptic digestion. This must come either from the splitting of arginyl bonds which have become *N*-terminal or from arginylarginine bonds which have become *C*-terminal during the digestion. Similarly, free arginine (0.4 mole/mole) and lysine (0.9 mole/mole) have been found in tryptic digests of lysozyme (309), although arginine was not detected with oxidized lysozyme (310). The free lysine may be derived from the *N*-terminal lysyl residue, and both an arginylarginyl (312) and lysylarginyl (310) sequence have been reported present.

The bonds split by trypsin in those peptides and proteins whose amino acid arrangement has been established are shown in Table I and Figures 2–10. From Table I it is apparent that, in general, the action of trypsin on various protein chains is in agreement with the specificities established on synthetic substrates. In the sections which follow only anomalous cases will be discussed.

(*a*) *Oxidized Insulin.* Trypsin has no action on fraction A (266) of oxidized insulin (Fig. 2); its action on fraction B (271) is shown in Figure 3. The splitting of a bond between the 16th and 22nd residues which did not involve arginine or lysine residues has been reported (102), but the possibility of contamination of the trypsin with

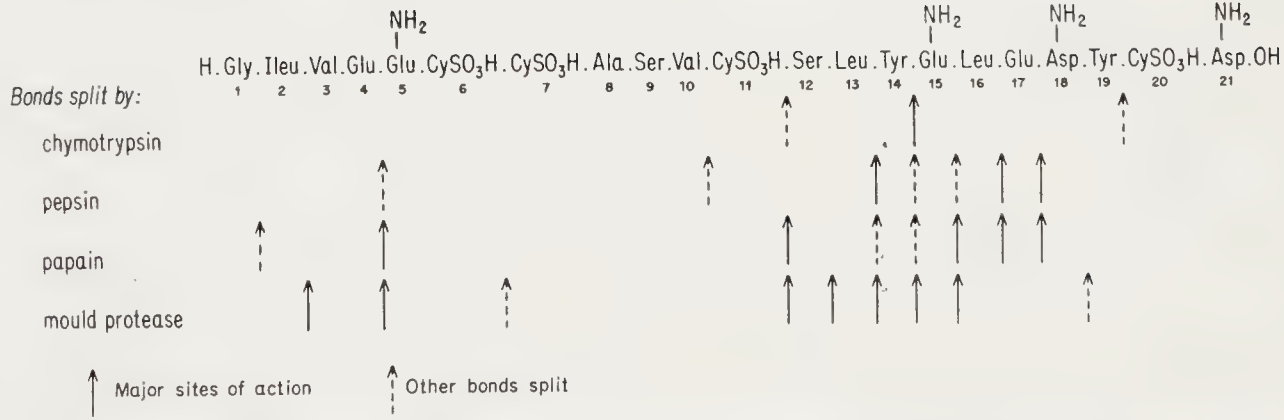


Fig. 2. The amino acid sequence of the A chain of oxidized bovine insulin showing the bonds hydrolyzed by proteolytic enzymes.

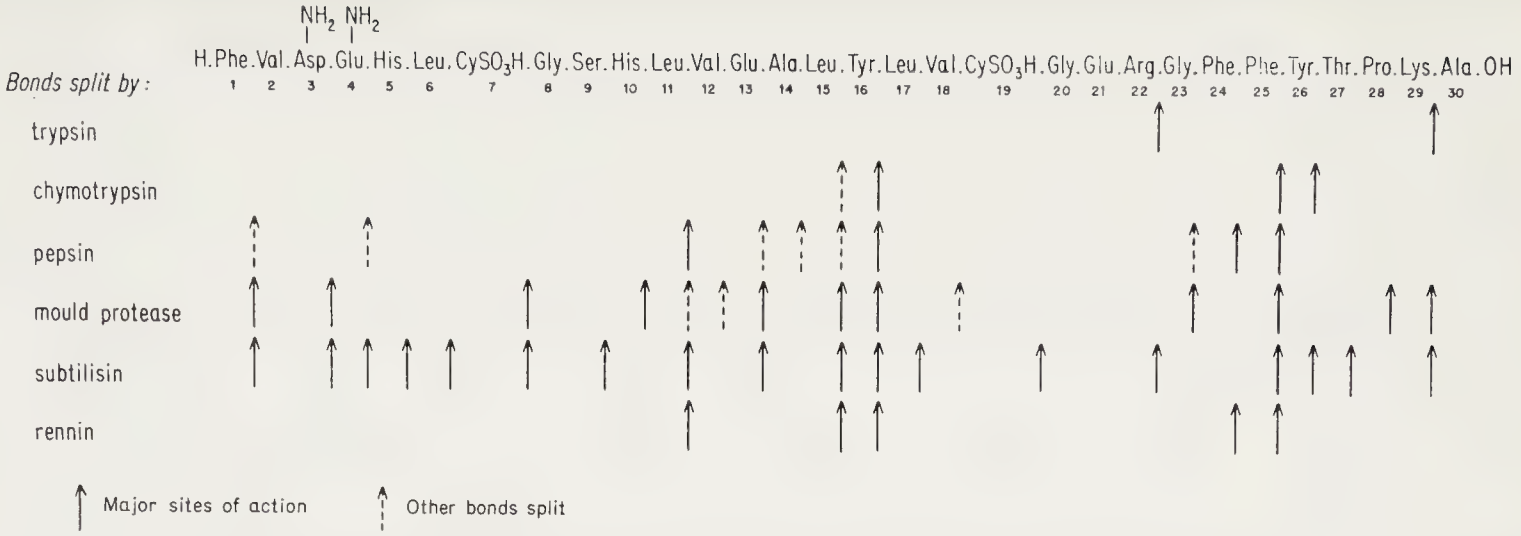

NH2 NH2
H.Phe.Val.Asp.Glu.His.Leu.CySO3H.Gly.Ser.His.Leu.Val.Glu.Ala.Leu.Tyr.Leu.Val.CySO3H.Gly.Glu.Arg.Gly.Phe.Phe.Tyr.Thr.Pro.Lys.Ala.OH
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Bonds split by:
trypsin
chymotrypsin
pepsin
mould protease
subtilisin
rennin
Major sites of action
Other bonds split

Fig. 3. The amino acid sequence of the B chain of oxidized insulin showing the bonds hydrolyzed by proteolytic enzymes.

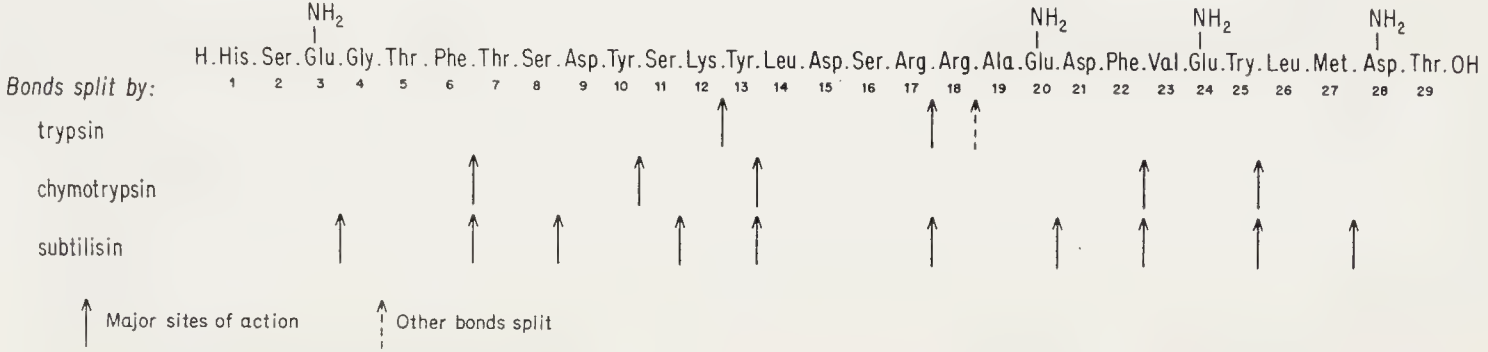

NH2 NH2 NH2 NH2
H.His.Ser.Glu.Gly.Thr.Phe.Thr.Ser.Asp.Tyr.Ser.Lys.Tyr.Leu.Asp.Ser.Arg.Arg.Ala.Glu.Asp.Phe.Val.Glu.Try.Leu.Met.Asp.Thr.OH
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
Bonds split by:
trypsin
chymotrypsin
subtilisin
Major sites of action
Other bonds split

Fig. 4. The amino acid sequence of glucagon showing the bonds hydrolyzed by proteolytic enzymes.

another protease was not excluded. A small amount of chymotrypsin impurity would account for splitting of the tyrosylleucyl bond.
16 17
In intact insulin, the action of trypsin is rather slow (49). At low enzyme/substrate ratios (140), the *C*-terminal alanine is liberated more rapidly than the heptapeptide. It would seem that the arginylglycyl bond may be more protected from enzymic attack when the disulfide bonds are intact.

(*b*) *Glucagon.* Glucagon (Fig. 4) contains two adjacent arginine residues -Arg.Arg.Ala (43). The arginylarginyl bond is split more
17 18 19
extensively (70%) than the arginylalanyl bond (20%) in 2 hr. digestion (44). If the arginylarginyl bond were split before the arginylalanyl bond, the α-amino group formed would be expected to inhibit hydrolysis of the arginylalanyl bond. However, the data showed that this bond was slowly attacked, reaching 80% liberation of free arginine after 50 hr. at 25° and pH 7.8.

In addition, other bonds, involving aromatic amino acid chains, were split. The scission of these linkages complicated the fractionation, and it is not clear whether their hydrolysis could be attributed to activity inherent in the trypsin or to contamination by another protease, such as chymotrypsin. In this connection, the recent report (209) of inherent chymotryptic activity in trypsin is of interest. This is supported by the close similarity in the chemical composition and sequence of amino acids of peptides isolated from around the active centers of trypsin and chymotrypsin and containing the diisopropyl phosphate residue of the inhibitor, diisopropyl fluorophosphate (224). The possibility of chymotrypsin contamination was not excluded, but all bonds susceptible to chymotrypsin were not attacked, and the addition of β-phenylpropionate, a chymotrypsin inhibitor, to the incubate did not prevent the two splits, 6–7 and 25–26, in question. Unfortunately, it is not clear if the enzyme was first incubated with the inhibitor before addition of the substrate. If not, the inhibition could not be expected to be complete.

(*c*) *Vasopressin.* Vasopressin contains one basic amino acid residue which is arginine in beef vasopressin (XII) and lysine in hog vasopressin. Trypsin releases glycine amide in both cases (83). It is of some interest that a prolyl residue precedes the basic group both here and in insulin and does not inhibit the enzyme, in contrast to the inhibition observed with a lysyl- or arginylprolyl bond (Table I).

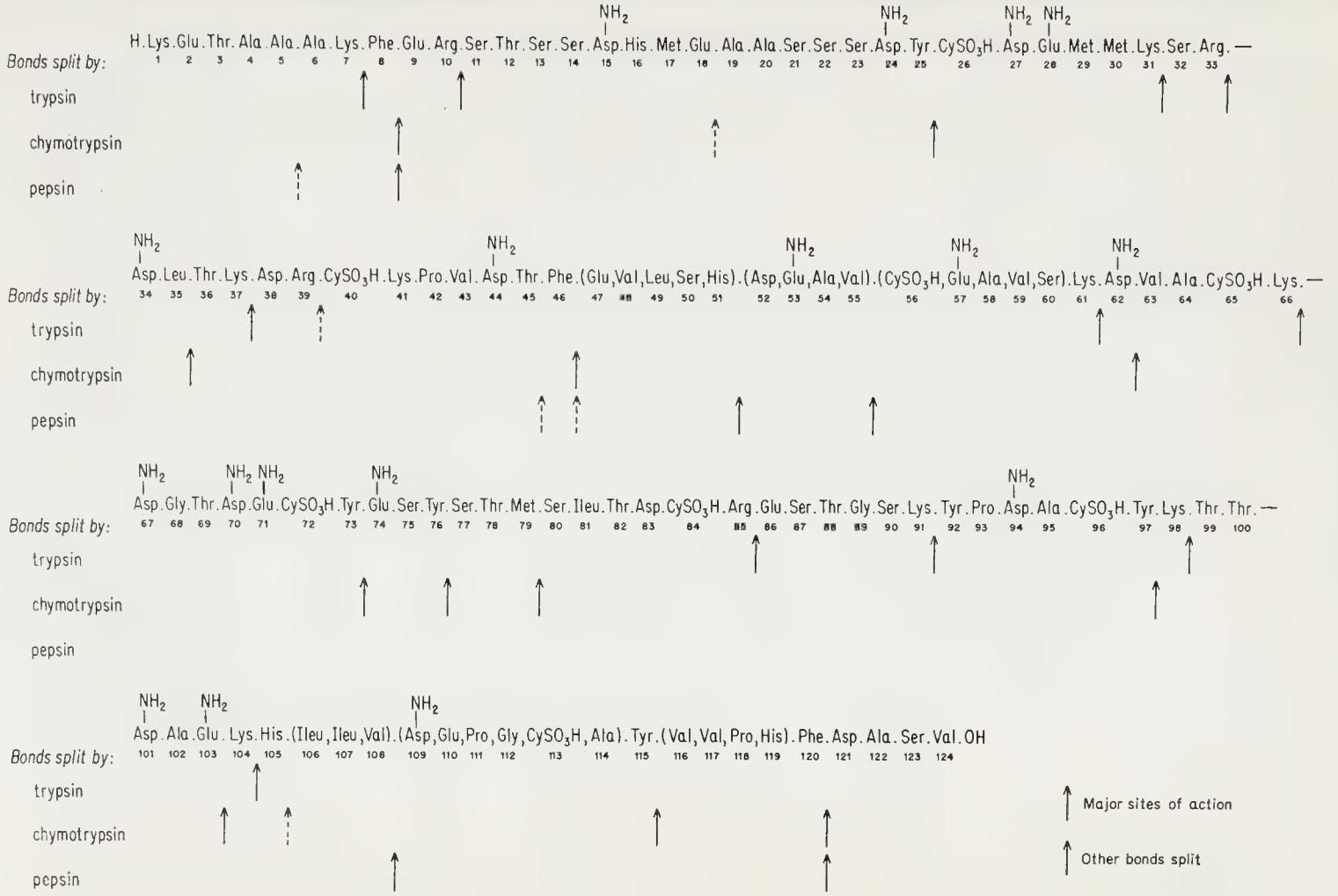


Fig. 5. A partial amino acid sequence of oxidized ribonuclease showing the bonds hydrolyzed by proteolytic enzymes.

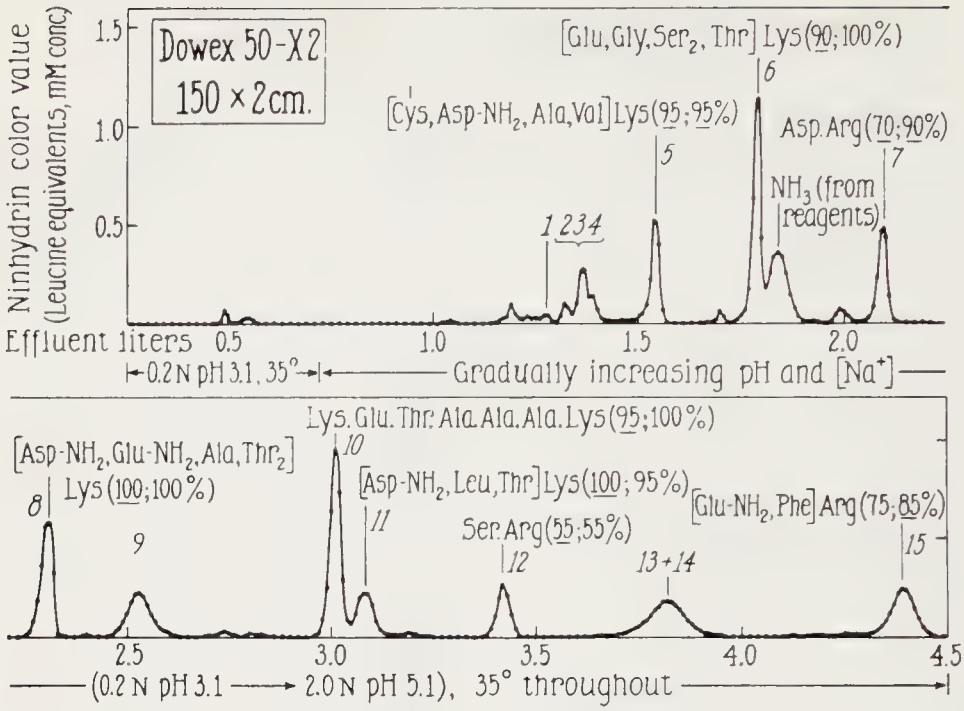
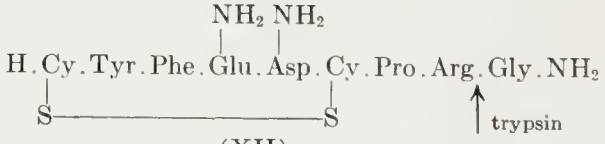

Ninhydrin color value (Leucine equivalents, mM conc.)
Dowex 50-X2
150 × 2cm.
[Glu, Gly, Ser2, Thr] Lys (90; 100%)
6
[Cys, Asp-NH2, Ala, Val] Lys (95; 95%)
5
Asp. Arg (70; 90%)
7
NH3 (from reagents)
1 2 3 4
Effluent liters 0.5 1.0 1.5 2.0
0.2 N pH 3.1, 35°
Gradually increasing pH and [Na+]
[Asp-NH2, Glu-NH2, Ala, Thr2] Lys (100; 100%)
8
9
Lys. Glu. Thr. Ala. Ala. Ala. Lys (95; 100%)
10
[Asp-NH2, Leu, Thr] Lys (100; 95%)
11
Ser. Arg (55; 55%)
12
13 + 14
[Glu-NH2, Phe] Arg (75; 85%)
15
2.5 3.0 3.5 4.0 4.5
(0.2 N pH 3.1 → 2.0 N pH 5.1), 35° throughout

Fig. 6. The peptides in a 20 hr. tryptic hydrolyzate of 200 mg. of oxidized ribonuclease. Chromatography was carried out on a column of sulfonated polystyrene and the effluent collected in 10 ml. fractions. Aliquots (0.5 ml.) were taken for analysis by the ninhydrin method. The figures in parentheses give the yield of each peptide after 3 and 20 hr. hydrolysis. The abbreviation Cys- represents a cysteic acid residue (reprinted with permission from ref. 152).

In corticotropin, discussed below, a lysylprolyl bond and an arginylprolyl bond were resistant.



(XII)

(*d*) *Oxidized Ribonuclease.* There are ten lysine and four arginine residues in a molecule of ribonuclease (153), and, if all bonds involving the carboxyl groups of these residues were quantitatively split by trypsin, fifteen fragments would be expected. However, neither the *N*-terminal lysyl residue (11) in this single polypeptide chain nor another lysyl sequence -Lys.Pro- was split (Fig. 5). Thus, thirteen
41 42

principal peptides were isolated by ion exchange chromatography (Fig. 6) in yields of 50–100% (152). The exacting work described by Hirs, Moore, and Stein is a model for future studies on other long chain proteins. The optimum time of hydrolysis was carefully studied and had a marked effect on the yield of three peptides, two of which were breakdown products of the third (residues 38–61) which contained a difficultly hydrolyzed -Arg.^{39}Cy^{40}SO$_3$H- bond. One other peptide (residues 67–85) decreased markedly with time, from 50% yield in 3 hr. to 15% in 20 hr., but the nature of the degradation was not established since no split products were found.

The thirteen principal peptides isolated accounted for all the amino acid residues of ribonuclease; in fact, they helped formulate the exact number of residues of two amino acids (152). The order in which these peptides were linked together in the original protein was determined from a study of the peptides produced by chymotryptic digestion (154).

An alternative method of specifically hydrolyzing oxidized ribonuclease with trypsin was studied by Redfield and Anfinsen (248). By masking the ϵ-amino groups of lysine with dinitrophenyl groups, the bonds involving these lysine residues were not cleaved by trypsin. Since there are only 4 moles of arginine per mole, it was anticipated that only five fragments would be produced; this was borne out by analysis. The bonds split were those shown in Figure 5 involving arginyl residues. The -Arg.^{39}Cy^{40}SO$_3$H- bond was cleaved much more slowly than the others. Of the five large peptides, the one containing no arginine must be *C*-terminal in oxidized ribonuclease, while the *N*-terminal peptide is identifiable by its *N*-terminal residue. The relative order of the other three fragments may be ascertained by isolation and identification from acid or enzyme hydrolyzates of arginine peptides, which can be readily detected by their specific color reactions (319). This general approach has given a partial structural formula for ribonuclease in agreement with that of Hirs *et al.* (154).

The amino acid sequence shown in Figure 5 has been compiled from the data published by both groups of workers (9,10,151,154, 154a).

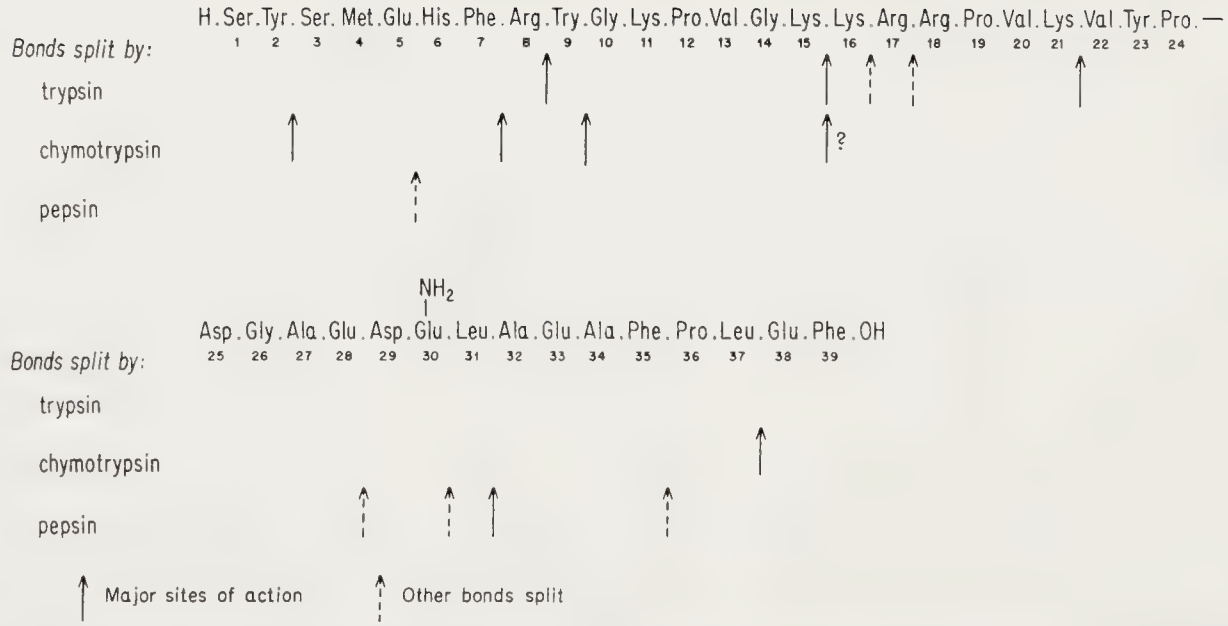


Fig. 7. The amino acid sequence of hog corticotropin showing the bonds hydrolyzed by proteolytic enzymes.

(*e*) *Corticotropin.* The sequence of hog corticotropin has been established by two groups of workers. The starting materials differ only in the number of amide groups since deamidation is possible during isolation. Hog corticotropin readily loses amide groups (79); thus, 18–22 hr. exposure to pH 9 at 25° results in the deamidation of the single glutaminyl residue (No. 30, Fig. 7), and this is complete in 2–3 days without loss of activity (282). This deamidation apparently occurred during the isolation of the corticotropin A of White and coworkers (337). The sequence of amino acids in hog corticotropin is given in Figure 7. The only area in which there is disagreement concerns the sequence of residues 25–28. Technical difficulties in the Edman degradation (Section V-B-1(a)), which was used extensively in these studies, may be the cause of this discrepancy (283). In one of the proposed structures (340), the sequence in residues 10–14 and 19–20 was not resolved. The portion of the molecule which seems essential for corticotropin activity resides in residues 1–24 (23,339), since limited pepsin digestion will remove eleven residues from the *C*-terminal end and dilute acid hydrolysis of the pepsin degradation product a few more residues without impairment of activity.

Splitting with trypsin rapidly inactivates the molecule, and an unusual grouping of four basic residues -Lys.Lys.Arg.Arg.Pro- leads to a variety of products derived from splits in this area. The -Lys.-Pro- and -Arg.Pro- bonds were resistant to trypsin. The yields of peptide fragments isolated by countercurrent distribution showed that bonds -Arg.Try-, -Lys.Lys-, and -Lys.Val- were at least 90% split (283). Partial splitting of the -Lys.Arg- and -Arg.Arg- bonds occurred to no more than 20%, giving 10–20% yields of four additional peptides. The more rapid splitting of the -Lys.Lys- bond will place a free amino group adjacent to the -Lys.Arg- bond and stabilize this to trypsin. Similarly, any molecules splitting first at the -Lys.Arg- bond will not be readily split subsequently at the -Arg.Arg- bond.

Thus, the following peptides, 93% weight recovery, were isolated due to splits in this region (compare with Fig. 7).

90% H.Try.Gly.Lys.Pro.Val.Gly.Lys.OH

10% max. H.Try.Gly.Lys.Pro.Val.Gly.Lys.Lys.OH

70% H.Lys.Arg.Arg.Pro.Val.Lys.OH

20% max. H.Arg.Arg.Pro.Val.Lys.OH

20% max. H.Arg.Pro.Val.Lys.OH

10% max. H.Lys.Arg.OH

Some free lysine or arginine might have been expected, but none was reported.

Sheep corticotropin shows a species difference with the sequence -Leu.Ala- replaced by -Ala.Ser- (198), and a different order in the 25–28 region where an -Ala.Gly.Glu.Asp- sequence has been reported. Digestion with trypsin took a different course in the -Lys.Lys.Arg.-Arg.- region, as indicated below.

$$\underset{15}{\text{-Lys}}\uparrow\underset{16}{\text{Lys}}\underset{17}{\text{Arg}}\uparrow\underset{18}{\text{Arg}}\uparrow\underset{19}{\text{Pro}}\underset{20}{\text{Val}}\underset{21}{\text{Lys}}\uparrow\underset{22}{\text{Val}}$$

H.Lys.Arg.OH

H.Arg.Pro.Val.Lys.OH

H.Lys.Arg.Arg.OH

Thus, no peptides contained the lysyllysyl bond, and no *N*-terminal arginylarginyl peptides were present, although a *C*-terminal arginylarginyl peptide was present. No H.Pro.Val.Lys.OH was reported, but splitting of the -Arg.Pro- bond was evidenced by the isolation of H.Lys.Arg.Arg.OH. Although the conditions of digestion (pH 9.3, 38°, 6 hr.) were different from those used with pig corticotropin (pH 7.8–9.0, 25°, 4 hr.), the different course of splitting by trypsin is surprising. This may be due to transpeptidation (192,332) reactions as in polylysine (Section IV-A-1).

(*f*) *Melanocyte-Stimulating Hormones.* Corticotropin, in addition to its corticotropic activity, possesses a melanocyte-stimulating activity which is about $^1/_{100}$ of that of a pure melanocyte-stimulating peptide (MSH) isolated from pig pituitaries (120,191,197). There are two active MSH peptides in pig pituitaries. The minor component, β-MSH, has been isolated by several groups of workers (25,120,191, 243), and the major component, α-MSH, has also been isolated in a

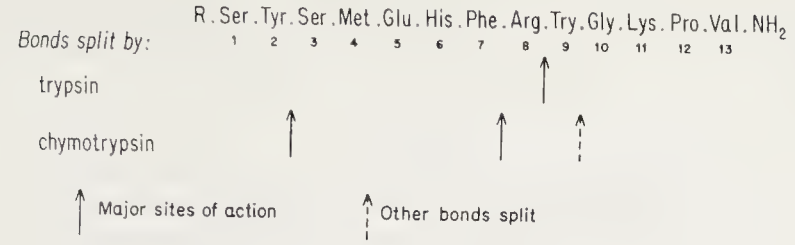

R . Ser . Tyr . Ser . Met . Glu . His . Phe . Arg . Try . Gly . Lys . Pro . Val . NH2
Bonds split by:
1 2 3 4 5 6 7 8 9 10 11 12 13
trypsin
chymotrypsin
Major sites of action
Other bonds split

Fig. 8. The amino acid sequence of α-melanocyte-stimulating hormone showing the bonds attacked by trypsin and chymotrypsin.

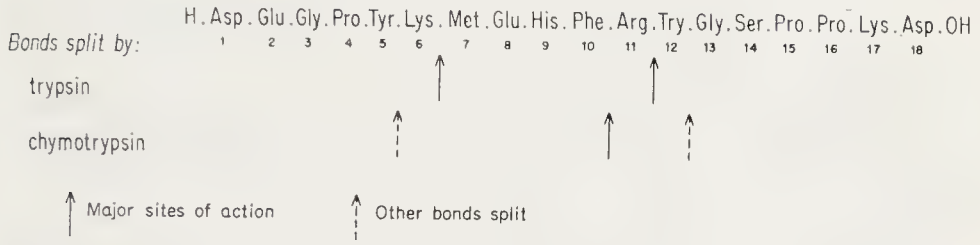

H . Asp . Glu . Gly . Pro . Tyr . Lys . Met . Glu . His . Phe . Arg . Try . Gly . Ser . Pro . Pro . Lys . Asp . OH
Bonds split by:
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
trypsin
chymotrypsin
Major sites of action
Other bonds split

Fig. 9. The amino acid sequence of porcine β-melanocyte-stimulating hormone showing the bonds attacked by trypsin and chymotrypsin.

pure condition (191). The amino acid sequences of α-MSH (139) and β-MSH (120,142) have been determined on minute amounts of material.

Thus, α-MSH (Fig. 8) has an identical sequence to the first thirteen residues of the *N*-terminal sequence of corticotropin, but its α-amino group is covered by an unknown substituent and it has a *C*-terminal amide group. β-MSH (Fig. 9) has residues 7–13 in the same sequence as α-MSH and corticotropin (residues 4–10) but different *N*- and *C*-terminal sequences.

Trypsin digestion of α-MSH gave only two fragments (139) corresponding to a split at the -Arg.Try- bond. The -Lys.Pro- bond was stable as in the case of corticotropin.

β-MSH with trypsin gave three fragments (120,142), the -Lys.Asp.-OH bond being resistant. All peptides were obtained in yields greater than 80%. Since the *C*-terminal -Lys.Ala.OH bond in insulin was split by trypsin, the stability of the -Lys.Asp.OH bond is apparently due to the combined effect of α- and γ-carboxyl groups of the aspartic acid, for there is a preceding prolyl residue in both. The resistance of the lysine bond in H.Tyr.Lys.Glu.OH but not in H.Tyr.Lys.Glu.Tyr.-

Fig. 10. The amino acid sequence of hypertensin showing the bonds split by trypsin and chymotrypsin.

OH has been reported (240). In ribonuclease (Fig. 5), -Arg.Glu- and
85 86
-Lys.Asp- bonds were readily split.
37 38

(*g*) *Hypertensin.* The action of rennin on serum protein gives rise to several pressor substances (235,278,290). The amino acid sequence of the major component from beef serum, hypertensin I, which is a decapeptide (98), is shown in Figure 10.

A similar sequence, but with isoleucine replacing the valine residue, has been determined (291) for a decapeptide from horse serum. It is converted into an octapeptide, called hypertensin II, by an enzyme occurring in plasma, with the release of histidinylleucine from the *C*-terminal position.

Trypsin splits only the -Arg.Val- bond in the molecule, as expected from its known specificity.

(*h*) *Other Proteins.* In studies of the amino acid sequence of other proteins, trypsin has given results in agreement with the established specificity. However, where the exact amino acid arrangement is not known, the effect of sequences on digestibility by trypsin cannot be evaluated.

Proteins which are being studied by tryptic digestion include papain (297), lysozyme (310), cytochrome *c* (324), protamine (216), chymotrypsinogen (224,256), trypsinogen (76,224), tobacco mosaic virus (111,273), and hemoglobin (162). In cytochrome *c*, a -Lys.Cys- bond is split by trypsin (324,326).

(*i*) *Conclusion.* In summing up, the specificity of trypsin can be said to be exacting in its requirement for a positively charged side chain of the appropriate dimensions that contributes a carboxyl group to the sensitive bond. However, the presence of the positively charged group does not necessarily make the succeeding bond sensitive. As we have seen, prolyl residues contributing an imino group

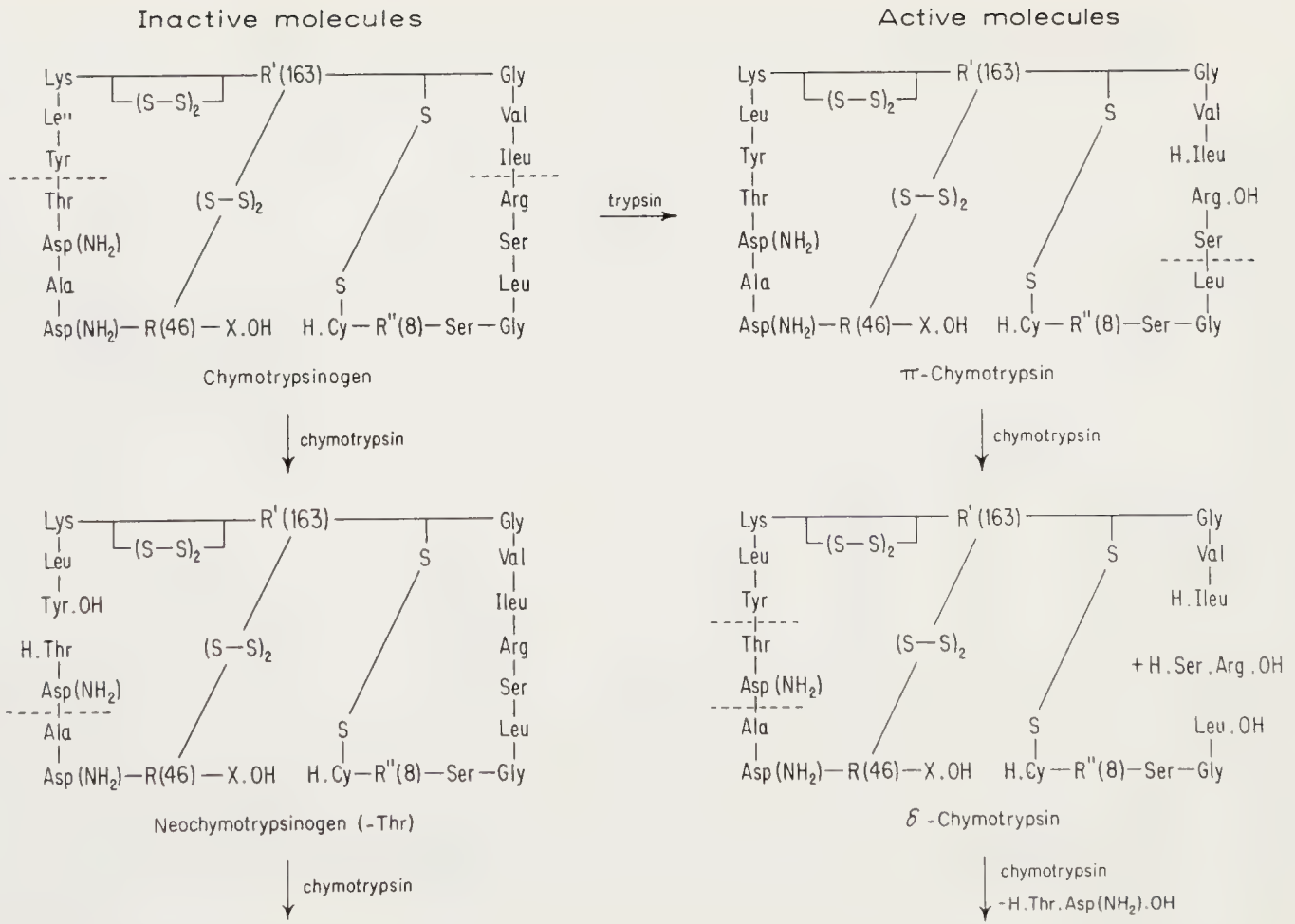
to the bond impart resistance to hydrolysis. Several basic residues adjacent to each other modify the sensitivity of bonds. Neighboring free α-amino groups or the combined effect of an α- and γ-carboxyl group may also prevent bond hydrolysis.

For studies of the amino acid sequences of peptides and proteins, and particularly as used in the case of oxidized ribonuclease, trypsin represents the most specific method of attack available at the present time. It must be remembered that, with native proteins, many bonds may be inaccessible to the enzyme but will be split when the molecule is unfolded by denaturation, oxidation, etc. Coupled with the use of other enzymes, particularly chymotrypsin, this method is capable of producing much information regarding amino acid sequences. The problem of fractionation of the peptides produced by tryptic digestion is a key one. Difficulties with DNP-peptides due to their insolubility and strong adsorption to paper or ion exchange columns may be reduced by the use of carbobenzoxy groups (12), which are readily removed by anhydrous hydrogen bromide in formic acid (26). The problem then becomes one of handling larger peptides. The choice of method, trypsin on oxidized protein or acylated oxidized protein, will be governed entirely by the size of the fragments and difficulties involved in fractionation. The upper limit of peptide size for good fractionation on Dowex 50-X2 columns is in the region of 20–30 residues (218).

When dealing with peptides containing cystine and methionine, without preliminary oxidation of the protein, additional factors such as their insolubility and tendency to oxidation must be considered.

2. Chymotrypsin

α-Chymotrypsin is one of a series of proteolytic enzymes that may be formed from the inactive precursor chymotrypsinogen. Activation is produced by small amounts of trypsin, but subtilisin (45) is also effective. Enzymic activity is associated with specific splitting by trypsin of only one peptide bond, -Arg.Ileu-, although chymotrypsinogen has twelve residues of lysine and four residues of arginine (128). From studies of the *N*- and *C*-terminal residues of chymotrypsinogen and the various decomposition products, a comprehensive scheme for the chemical changes involved in the activation of chymotrypsinogen has been determined and is summarized in recent papers (223,224,256), as set out below and illustrated in Figure 11.


Inactive molecules
Active molecules
Lys
Leu
Tyr
Thr
Asp(NH2)
Ala
Asp(NH2)—R(46)—X.OH
R'(163)
(S—S)2
Gly
Val
Ileu
Arg
Ser
Leu
H.Cy—R"(8)—Ser—Gly
S
trypsin
Chymotrypsinogen
π-Chymotrypsin
chymotrypsin
Tyr.OH
H.Thr
H.Ileu
Arg.OH
Neochymotrypsinogen (-Thr)
δ-Chymotrypsin
+H.Ser.Arg.OH
Leu.OH
-H.Thr.Asp(NH2).OH

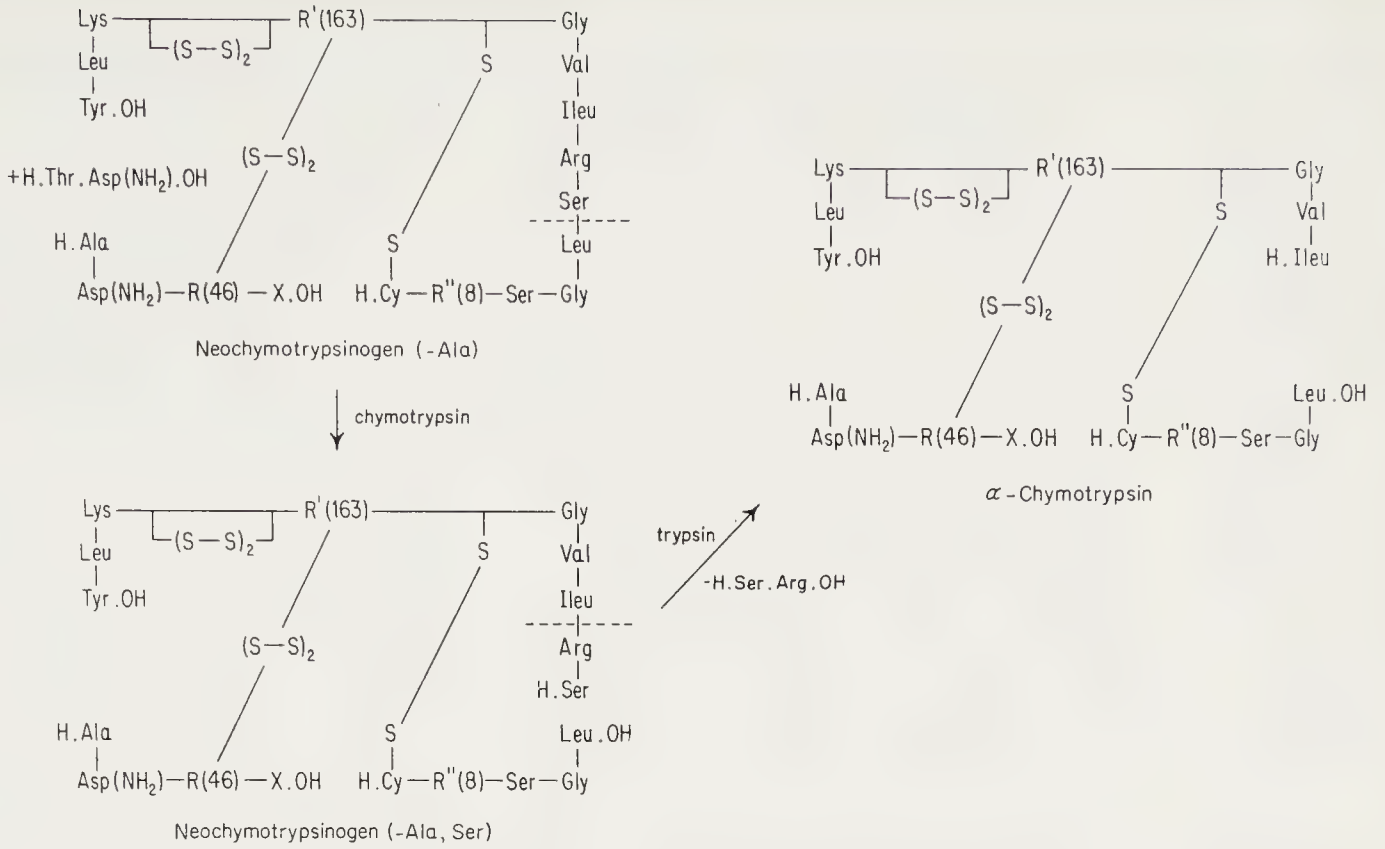
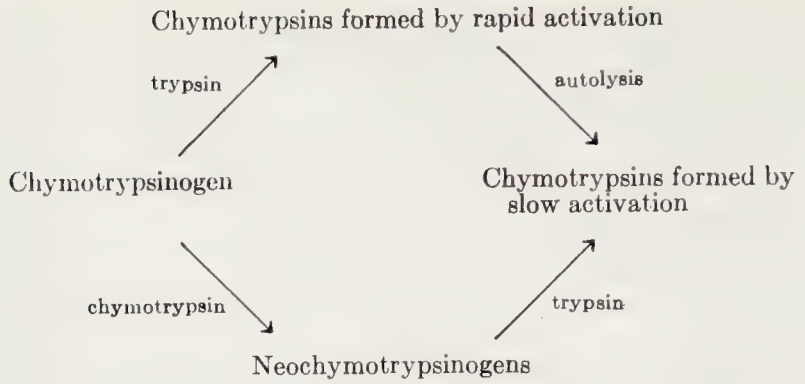


Fig. 11. Chemical changes associated with the activation of chymotrypsinogen. The figure enclosed in brackets denotes the number of amino acid residues in this section of the polypeptide chain. Chymotryptic activity is obtained as soon as the -Arg.- Ileu- bond is split by trypsin. The further action of chymotrypsin gives other active molecules. All neochymotrypsinogens are activated by tryptic cleavage of the -Arg.Ileu- bond, but only the activation of neochymotrypsinogen (Ala,Ser) is shown. The abbreviations in brackets indicate *N*-terminal residues of alanine and serine and serve to differentiate the different neochymotrypsinogens.



The neochymotrypsinogens and chymotrypsins illustrated in Figure 11 are not the only members of these families of related proteins. Other possibilities have been visualized (256), and there is indirect evidence for the existence of some of them. The accumulation of the information shown in Figure 11 is the result of many years of work in several laboratories, notably those of Desnuelle and Neurath, and is a good illustration of the steady progress in the improvement in techniques over that period.

The *C*-terminal amino acid in chymotrypsinogen was unreactive to carboxypeptidase or hydrazinolysis (224). However, with urea-denatured chymotrypsinogen, amino acids are liberated by carboxypeptidase and (Ala,Val).Leu has been reported to be the *C*-terminal sequence (118). However, conflicting data have been reported (213,223), and the *C*-terminal residue has been shown as X.OH in Figure 11.

The pH range for chymotryptic activity is between 7 and 9 and varies somewhat according to the substrate (128). Some synthetic substrates—e.g., benzoyl-L-tyrosinamide—are maximally hydrolyzed at pH 7.8.

The enzyme is more stable than trypsin at the pH for optimum activity, but in concentrated solution at pH 7.8 autolyzes without loss of activity into two new forms, β- and γ-chymotrypsin.

From studies with synthetic substrates, the specificity of chymotrypsin has been shown to be somewhat broader than that of trypsin. In general, peptide bonds hydrolyzed by chymotrypsin involve the carboxyl groups of amino acids possessing side chains with aromatic character—i.e., tyrosine, phenylalanine, or tryptophan. In addition, methionine and leucine compounds are attacked, but at a much slower rate (128,227).

Chymotrypsin has been used for the hydrolysis of peptides and proteins prior to isolation of peptides for amino acid sequence studies. The information so far accumulated on the types of bond split by this enzyme (Table II) supports the general preference for bonds involving phenylalanyl and tyrosyl linkages; but in some cases this type of bond was resistant, while in others different types of bonds were attacked.

In the activation of chymotrypsinogen by trypsin, which also involves autolysis or the action of chymotrypsin on chymotrypsinogen, it is clear that two bonds, -Leu.Ser.- and -Asp(NH_2).Ala-, which do not involve side chains of aromatic character, are split by chymotrypsin, in addition to the expected hydrolysis of a -Tyr.Thr- bond. This is the only splitting involving an aromatic residue, although the protein contains four tyrosine, six phenylalanine, and six tryptophan residues. Again, the stability of native proteins to enzyme digestion is in evidence.

(*a*) *Oxidized Insulin.* The glycyl chain of oxidized insulin contains two tyrosyl residues. Both bonds involving these residues are hydrolyzed (266). The -$\overset{14}{\text{Tyr}}.\overset{15}{\text{Glu}}(NH_2)$- bond is hydrolyzed much more rapidly and completely than the -$\overset{19}{\text{Tyr}}.\overset{20}{\text{Cy}}SO_3H$- bond. Thus, after 5 min. only the 14–15 bond is split, and only two peptides are present; whereas, after 16 hr., four additional components may be isolated (143), corresponding to the splitting of the 19–20 bond and also, surprisingly, a -$CySO_3H$.Ser- (11–12) bond (Fig. 2).

The partial splitting at the 11–12 and the 19–20 bonds has the disadvantage of increasing the complexity of the digests, making subsequent fractionation more difficult. Similar complications occur whenever partial modification of a residue takes place. For example, conversion of a portion of the tyrosine residues to chlorotyrosine during oxidation resulted in duplication of each tyrosine-containing peptide. Partial deamidation during manufacture, as has been demonstrated (54,55,133) for several proteins, can also cause duplication into amidized and deamidized forms (268). Another complication encountered with *N*-terminal glutaminyl peptides is their ability to lose ammonia from the glutaminyl residue to form the corresponding pyroglutamyl peptide, as in oxidized insulin (266,268) and ribonuclease (154).

The splitting of the -$\overset{11}{\text{Cy}}SO_3H.\overset{12}{\text{Ser}}$- bond by chymotrypsin was un-

TABLE II
Action of Chymotrypsin on Various Proteins

Peptide or protein	Bonds readily split and involving Tyr, Phe, or Try	Bonds partially split and involving Tyr, Phe, or Try	Resistant bonds involving Tyr, Phe, or Try	Other bonds split
Oxidized insulin				
A chain	Tyr. Glu(NH_2) 14 15	Tyr. $CySO_3H$ 19 20		$CySO_3H$. Ser 11 12
B chain	Phe. Tyr 25 26	Tyr. Leu 16 17	Leu. Tyr 15 16	Phe. Phe 24 25
		Try. Thr 26 27		
Glucagon	Phe. Thr 6 7	Tyr. Ser 10 11		
	Phe. Val 22 23	Tyr. Leu 13 14		
	Try. Leu 25 26			

Oxidized ribonuclease	Phe. Glu 8 9	Tyr. $CySO_3H$ 25 26			Tyr. Pro 92 93	Glu. Ala 18 19
	Phe. ? 46 47	Tyr. $Glu(NH_2)$ 73 74				Leu. Thr 35 36
	Phe. Asp 120 121	Tyr. Ser 76 77				Asp(NH_2). Val 62 63
		Tyr. Lys 97 98				Met. Ser 79 80
		Tyr. ? 115 116				Glu(NH_2). Lys 103 104
						His. Ileu or Val 105 106
Corticotropin	Phe. Arg 7 8	Tyr. Ser 2 3			Tyr. Pro 23 24	Lys. Lys 15 16
	Try. Gly 9 10				Phe. Pro 35 36	Leu. Glu 37 38
α-MSH	Phe. Arg 7 8	Tyr. Ser 2 3	Try. Gly 9 10			
β-MSH	Phe. Arg 10 11		Try. Gly 12 13	Tyr. Lys 5 6		
Hypertensin	Phe. His 8 9	Tyr. Val 4 5				

expected. Control experiments, using chymotrypsin which had previously been incubated with the specific inhibitor di-isopropyl fluorophosphate, showed no splitting of the glycyl chain. Splitting of the same bond by α-chymotrypsin also occurred in a smaller peptide comprising residues 1–13 of the glycyl chain, but a simple model, the ammonium salt of *N*-acetyl-L-cysteic acid carboxamide, was not hydrolyzed (219).

There is some evidence (227) that a negative charge near the sensitive bond may inhibit splitting by chymotrypsin. This is not substantiated by the splitting of the -$CySO_3H$.Ser- and -Tyr.$CySO_3H$- bonds, which have strong negative charges because of the adjacent —SO_3^- group, or of other bonds such as -Phe.Asp- (Table II).

In the phenylalanyl chain of insulin, the bond adjacent to the *N*-terminal phenylalanyl residue was not split, suggesting inhibition by the nearby positively charged group. The -Phe.Phe.Tyr- grouping
24 25 26
in this peptide might be expected to be split in a complex manner. The main splitting occurred after the tyrosine residue (bond 26–27) (271). The stability of the *C*-terminal -Phe.Phe- group in the resulting peptide suggests that an adjacent negative charge from an α-carboxyl group retards hydrolysis of bonds normally susceptible.

(*b*) *Oxidized Ribonuclease.* The action of chymotrypsin was less specific than trypsin in the case of ribonuclease, as evidenced by the lower yields of peptides obtained by ion exchange chromatography (154). The isolated peptides accounted for 151 residues, instead of the 124 residues accounted for in peptides from tryptic hydrolyzates. Obviously, certain portions of the peptide chain appeared in more than one of the peptide fragments. The increased complexity of the digest was reflected in the small amounts of impurities (rarely greater than 15%) that were present in most of the peak fractions. These impurities did not complicate the interpretation of amino acid analyses of the fractions, but they do emphasize the difficulties encountered in fractionation of peptide mixtures obtained by less specific methods of hydrolysis. The hydrolyzates investigated in the case of ribonuclease were prepared by 24 hr. digestion at pH 7. Shorter times gave additional peptides, since only partial splitting of certain bonds had occurred, and smaller amounts of the split products were obtained. The principal peptides were obtained in yields of 40–80%. The bonds split by chymotrypsin are shown in Figure 5.

If splitting of any other bonds reached 10%, they would have been detected. For example, a peptide with the approximate composition $CySO_3H$.Glu.Asp.Met_2. estimated to be present in about 20% yield, may be indicative of partial splitting of a -Met.Lys- bond.
30 31

Only one bond containing tyrosine or phenylalanine, -Tyr.Pro-, was completely resistant to hydrolysis. The peptide bonds split are shown in Table II. The 15% splitting of a -Lys.Phe- bond may have been due to the presence of a trace of trypsin in the chymotrypsin.
92 93 7 8

(*c*) *Glucagon.* No anomalies were observed in the hydrolysis of this polypeptide by chymotrypsin (Figure 4), and all bonds were at least 80% split.

(*d*) *Corticotropin.* The action of chymotrypsin on corticotropin (283) is shown in Figure 7; -Tyr.Pro- and -Phe.Pro- bonds were resistant.
23 24 35 36

The presence of a positively charged group in a -Phe.Arg.- sequence did not prevent quantitative splitting. Neither the absence of an aromatic side chain nor an adjacent negative charge prevented quantitative cleavage of a -Leu.Glu- sequence. However, neither a -Met.Glu- nor a -Leu.Ala- sequence was split. The splitting of a -Lys.Lys- bond by chymotrypsin is definitely unexpected and may possibly be due to contamination by trypsin.
7 8 37 38 4 5 31 32 15 16

(*e*) *Melanocyte-Stimulating Peptides.* These peptides were split in the expected positions, as shown in Figures 8 and 9. The bond involving a tryptophan residue was not completely split under the conditions used (120,142,191). Thus, the -Try.Gly- sequence in α-MSH was not completely split in 30 min. at 30° and pH 8.2, but after 16 hr., digestion was apparently complete. The corresponding -Try.Gly- sequence in β-MSH was also only partially hydrolyzed (120,142), and, even after 18–24 hr. at pH 8 and 38°, some of the larger peptides (residues 12–18) could be isolated (120).
9 10 12 13

The -Tyr.Lys- bond (Fig. 9) was only partially split in 20 min. at 30° and pH 8 by chymotrypsin, and three peptides, two of which were derived from the third, were isolated from the 1–10 sequence (142). The presence of the basic group of the lysyl side chain is un-
5 6

likely to have had much effect on the rate of splitting in this case, since the -Phe.Arg- sequence in both α- and β-MSH is rapidly and completely hydrolyzed. The preceding prolyl residue may be involved.

As in the related corticotropin sequence, the -Met.Glu- sequence is resistant to chymotrypsin.

(*f*) *Conclusion.* Chymotrypsin is sufficiently specific in its action on long-chain substrates to be valuable for the selective degradation of proteins. If a prolyl residue follows the amino acid with aromatic side chain, the peptide bond will be resistant to hydrolysis.

Adjoining α-amino or α-carboxyl groups markedly retard the rate of hydrolysis. Other basic or acidic side chains have less effect. Moreover, the residue contributing the carboxyl group to the peptide bond was not restricted to aromatic amino acids only, since bonds involving a wide range of amino acids show some degree of splitting (see Table II).

3. Other Enzymes

Several other enzymes have been used in the fragmentation of peptide and protein molecules, but none of these has been sufficiently specific to merit detailed discussion. Some brief remarks will serve to focus attention on their potentialities. Although relatively nonspecific, these enzymes are extremely useful in obtaining small fragments from native proteins in order to yield information on amide distribution (268), identity of amides (whether asparaginyl or glutaminyl, rather than isoasparaginyl or isoglutaminyl, residues are present (187)), disulfide bridges (258), or sequences involving particular amino acid residues that may be readily detected by specific color reactions (e.g., arginine-containing sequences) (248). Further digestion of large peptides from tryptic digests may be useful as a source of simple peptides for identification (271).

(*a*) *Pepsin.* Below pH 5, pepsinogen is converted to pepsin autocatalytically according to the following reaction

$$\text{Pepsinogen} \longrightarrow \text{Pepsin} + \text{Inhibitor} + \text{Miscellaneous peptides}$$

The amino acid compositions of pepsinogen, pepsin, and the pepsin inhibitor are known (327,328); and, from a study of the *N*-terminal and *C*-terminal sequences, it is apparent that pepsin is formed from the *C*-terminal end of pepsinogen since both pepsin and pepsinogen have

identical *C*-terminal sequences but different *N*-terminal sequences (147,328).

There is evidence to show that pepsin is not so homogeneous as trypsin and chymotrypsin (128). This may account, in part, for the wide spectrum of bonds attacked by this enzyme. From its effect on

TABLE III
Action of Pepsin on Various Proteins

	Bonds split		Resistant bonds involving amino group of tyrosine or phenylalanine	
Oxidized insulin				
A chain	Glu.Glu(NH_2) 4 5	Glu(NH_2).Leu 15 16	Asp(NH_2).Tyr 18 19	
	Leu.Tyr 13 14	Leu.Glu 16 17		
	Tyr.Glu(NH_2) 14 15	Glu.Asp(NH_2) 17 18		
B Chain	H.Phe.Val 1 2	Leu.Tyr 15 16		
	Glu(NH_2).His 4 5	Tyr.Leu 16 17		
	Leu.Val 11 12	Gly.Phe 23 24		
	Glu.Ala 13 14	Phe.Phe 24 25		
	Ala.Leu 14 15	Phe.Tyr 25 26		
Corticotropin	Glu.His 5 6	Glu.Ala 33 34	H.Ser.Tyr 1 2	
	Glu.Asp 28 29	Phe.Pro 35 36	His.Phe 6 7	
	Asp.Glu(NH_2) 29 30	Glu.Phe.OH 38 39	Val.Tyr 22 23	
	Glu(NH_2).Leu 30 31			
Oxidized ribonuclease	Ala.Ala 5 6	Phe.?	Lys.Phe 7 8	Lys.Tyr 91 92
	Phe.Glu 8 9	Bonds 51–52, 55–56, 108–109	Ser.Tyr 24 25	$CySO_3H$.Tyr 96 97
	Thr.Phe 45 46		$CySO_3H$.Tyr 72 73	Ala.Tyr 114 115
	Phe.Asp 120 121		Ser.Tyr 75 76	?.Phe 119 120

synthetic substrates, pepsin was shown to attack bonds involving the amino group of amino acids with side chains of aromatic character.

A typical substrate is carbobenzoxyglutamyltyrosine. The presence of an amino group adjacent to the sensitive bond inhibited the action of the enzyme, and, if one of the carboxyl groups in this substrate was masked by amide formation, activity was also lowered, and ceased when both carboxyls were masked (27).

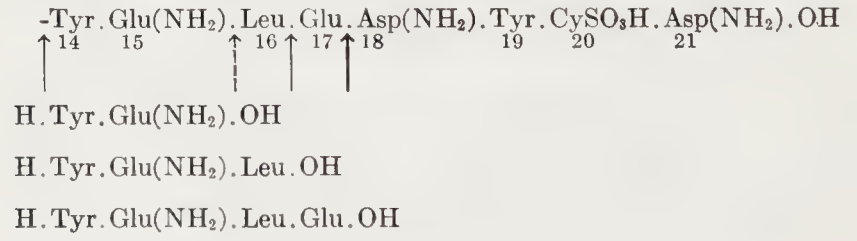
Pepsin is optimally active on most synthetic substrates at pH 4, but for protein substrates and synthetic substrates with consecutive aromatic residues, such as acetylphenylalanylphenylalanine, the optimum pH is near 2. In this acid pH region, pepsin is unstable due to autolysis. Maximum stability is at pH 5–5.5, and the enzyme is rapidly denatured above pH 6.

The action of pepsin has been studied in some detail on the A (266) and B (271) chains of oxidized insulin (Figs. 2 and 3), on corticotropin (23) (Fig. 7), and on oxidized ribonuclease (17) (Fig. 5).

Table III shows the bonds which are split by pepsin, together with those that should have been attacked on the basis of the specificity established with synthetic substrates. It has not been profitable to try to distinguish between bonds readily split and those partially split, since in many cases splitting of certain bonds modifies the behavior of adjacent susceptible bonds. Moreover, the data for ribonuclease are not complete, since the exact sequences of some residues are still unknown.

However, it is obvious from inspection of Table III that many bonds not involving aromatic residues have been split, and that as many bonds of the required type have proved resistant. It is apparent that either a variety of enzymes is involved or the essential structural configuration required for pepsin attack is still not clearly defined.

The method of indicating enzyme attack in the relevant figures does not indicate the true complexity of the digests. For example, in the A chain of oxidized insulin, the pepsin digestion gives rise to seven peptides from an attack on four bonds shown below



H.Leu.Glu.Asp(NH_2).Tyr.CySO_3H.Asp(NH_2).OH

H.Glu.Asp(NH_2).Tyr.CySO_3H.Asp(NH_2).OH

H.Asp(NH_2).Tyr.CySO_3H.Asp(NH_2).OH

H.Leu.Glu.OH

To some extent, the complexity of this mixture will be due to the inhibition of pepsin by adjacent amino groups. A molecule splitting first at bond 16–17 will not be readily split at the adjacent 17–18 bond, and so on.

In spite of the low specificity of pepsin, one large *N*-terminal peptide containing thirteen amino acid residues was isolated from the A chain, and, together with the peptides identified in partial acid hydrolyzates, this single peptide was sufficient to deduce the sequence in the A chain (266).

Hog corticotropin behaves in a similar manner, giving three large active fragments (23) comprising residues 1–28, 1–30, and 1–31. A large number of small peptides is derived from the readily digested tail of the corticotropin (23,195).

In contrast to the extensive digestion of oxidized ribonuclease, the native enzyme can be treated with pepsin under conditions such that only one peptide bond is hydrolyzed with the release of a tetrapeptide from the *C*-terminal end of the chain and formation of an enzymatically inactive protein (8).

(*b*) *Papain.* This enzyme may now be crystallized in a high state of purity from the extracts of commercial dry papaya latex (177). The enzyme depends on one sulfhydryl group for activity (295) and, provided a reducing agent (e.g., cysteine or cyanide) and a metal-complexing agent (e.g., ethylenediaminetetraacetate) are present, reproducible activities are obtained.

The protein has a molecular weight of about 20,500 and contains approximately 180 amino acid residues, but, by digestion with leucine aminopeptidase, as many as two-thirds of the amino acid residues may be removed from the *N*-terminal end without loss of enzymic activity (148).

The pH range for maximum enzymic activity is 5–7.5, but at the higher pH values synthetic and transfer reactions are possible (117, 177).

Partially purified papain was shown (27) to attack many types of synthetic amide and peptide substrates. The crystalline enzyme

exhibits similar properties (177), and synthetic substrates for trypsin, pepsin, chymotrypsin, carboxypeptidase, and peptidases are all attacked, although at very different rates. Benzoyl-L-arginineamide has so far proved to be the most sensitive substrate. The attack of the enzyme on Fraction A of oxidized insulin exhibited a broad hydrolytic action rather similar to that of pepsin, although the relative intensities of splitting of the various bonds were markedly different. The sites of attack are shown in Figure 2. It is of interest that the splitting of the -CySO_3H.Ser- bond in intact Fraction A does not take place in the isolated peptide comprising residues 1–13, in which the -Ser.Val- bond was attacked instead. This is another illustration of the importance of factors other than the nature of the residues involved in the hydrolyzable bond.

(*c*) *Mold Protease and Subtilisin.* A mold protease isolated in crystalline form from culture filtrates of *A. oryzae* was many times as active as papain in the digestion of casein and hemoglobin (66). This enzyme was found to carry the digestion of the chains of oxidized insulin further than any of the other enzymes so far considered (268). The bonds attacked are shown in Figures 2 and 3.

Subtilisin was crystallized by Guntleberg and Ottesen (130) from a medium in which a strain of *B. subtilis* had grown. This proteolytic enzyme was originally detected by its ability to effect limited hydrolysis of native ovalbumin with the formation of another crystallizable protein, plakalbumin (199), and the release of a hexapeptide, Ala.Gly.Val.Asp.Ala.Ala (230).

The specific nature of this cleavage is remarkable in view of the variety of peptide bonds attacked in other proteins. In general, it has a wide specificity like that of mold protease, and there are similarities in crystalline form and conditions of crystallization between these two enzymes. However, they have different isoelectric points (130) and are probably not identical.

Subtilisin attacked oxidized oxytocin (XIII) at the bonds shown (325).

H.CySO_3H.Tyr.Ileu.Glu(NH_2).Asp(NH_2).CySO_3H.Pro.Leu.Gly.NH_2

(XIII)

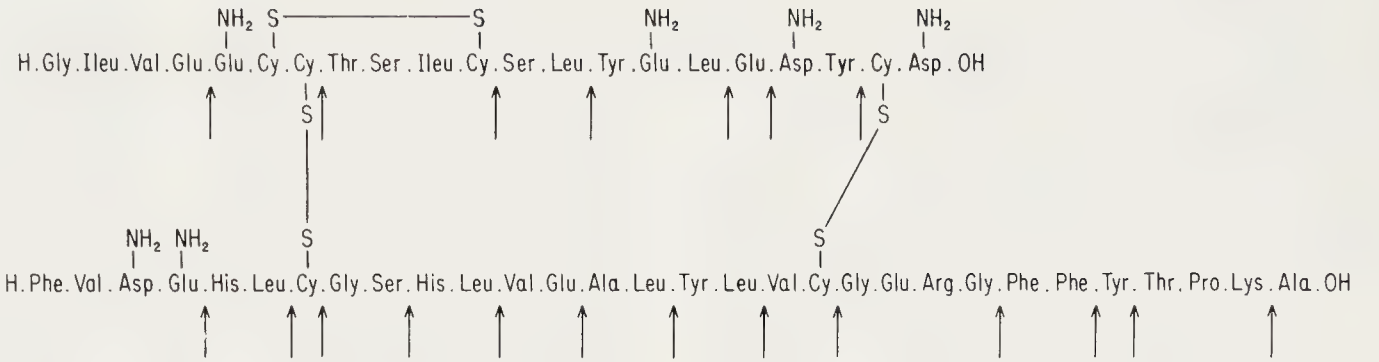


Fig. 12. The structure of pork insulin showing the bonds hydrolyzed by subtilisin.

In the B chain of oxidized insulin (Fig. 3) (323), 18 of a total of 29 bonds were split. In native pork insulin (212), only 13 bonds in the B chain were hydrolyzed, indicating an effect of structural factors on the course of proteolysis. Figure 12 shows the points of fission in pork insulin; those in glucagon appear in Figure 4. Some information on the effect of subtilisin on ribonuclease has been summarized (10).

No generalizations regarding the specificity of this enzyme seem worthwhile. The preference for bonds involving the carboxyl groups of leucine in insulin was not apparent (286) with glucagon, and it appears that all types of residues may form part of the susceptible bond, with the possible exception of proline.

(*d*) *Lung Proteinase I and Rennin.* A purified proteinase isolated from bovine lung, for which no synthetic substrate has so far been found, hydrolyzed the B chain of oxidized insulin at the same bonds as did pepsin (67).

Crystalline rennin has also been studied in its action on this substrate (103) and found to hydrolyze some of the bonds attacked by pepsin. Adequate controls were studied to exclude the possibility of pepsin contamination, and the sites of action are shown in Figure 3.

(*e*) *Thrombin.* The conversion of fibrinogen to fibrin by thrombin is an example of limited proteolysis involving only two or three bonds out of about 3000 bonds in fibrin, resulting in the release of two peptides which have been characterized (18,30). Both peptides contain arginine, and, since thrombin hydrolyzes synthetic substrates such as toluenesulfonyl-L-arginine methyl ester (284), it was concluded that arginyl bonds in fibrinogen had been split. However, the lysine in one of the peptides could be part of the sensitive bond, since both lysine ethyl ester and the lysylalanine bond in the B chain of oxidized insulin are substrates for thrombin (95). No proteolytic activity of thrombin on ovalbumin or rabbit myosin could be detected (18).

B. CHEMICAL METHODS

A chemical method for the selective cleavage of a peptide chain must utilize an amino acid side chain that exhibits a distinctive type of reactivity. Thus, the simple aliphatic side chains of glycine, alanine, valine, and the leucines are obviously not suitable.

The various reactive side chains are indicated in Table IV. Proline has been shown together with its imino and carboxyl groups. It (and

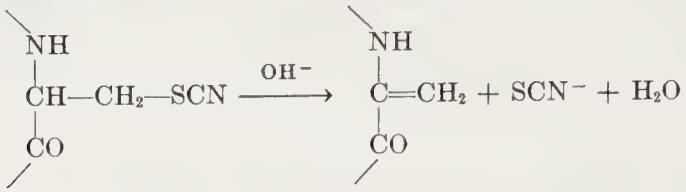
hydroxyproline) is exceptional in not possessing an —NH— group in the peptide chain capable of hydrogen bonding. The geometry of the closed pyrrolidine ring suggests discontinuities in the stereochemical structure of protein chains (93,202). A chemical attack on proline residues, if one could be devised, might be worthy of study.

Attempts to exploit the cystine or cysteine side chains formed by reduction of cystine for specific cleavages have already been dis-

TABLE IV
Amino Acids with Reactive Side Chains

Amino acid	Side chain
Cysteine	$-CH_2SH$
Cystine	$-CH_2S-SCH_2-$
Methionine	$-CH_2 \cdot CH_2 \cdot SCH_3$
Serine	$-CH_2OH$
Threonine	$-CHOH$ $\quad\quad\quad

cussed (Sections III-B and IV-A-1). Another possibility (302) is the conversion of these residues to —CH_2.SCN groups followed by alkaline degradation to dehydroalanine (α-aminoacrylic) residues which will give reactive points in the peptide chain



which should be readily split by dilute acid or alkali, or possibly enzymically with dehydropeptidases.

The attempts to employ the hydroxyamino acids are described in more detail in Section IV-B-1. The basic amino acids have only been used in conjunction with trypsin as previously discussed (Section IV-A-1). Attempts to use the aromatic amino acids have been made, based on the photolysis of peptide bonds near these residues when the protein is irradiated with light of a suitable wavelength. Much of this work is of an empirical nature and concerned with alterations in the properties of the proteins concerned, often with enzymic or immunological properties. Several reviews of the effects on proteins are available (80,101,210).

Experiments designed to test the possibility of specific cleavages are relatively few. Although the irradiation of proteins results in the breaking of peptide bonds, in the case of lysozyme (285) no low molecular weight peptides or amino acids could be detected.

The action of bromine water on proteins discussed in Section IV-B-2 is another cleavage reaction that may involve tyrosine residues.

Tryptophan is rapidly destroyed by acids, but whether the peptide chain breaks at tryptophan residues as rapidly as the side chain is destroyed is not known. Oxidation of tryptophan with performic acid is known to involve the uptake of three atoms of oxygen with darkening, but no peptide bond breakdown occurs (Section III-A). It is possible, however, that a suitable method might be devised to utilize the oxidized tryptophan residues for a more rapid cleavage of the peptide chain at that point.

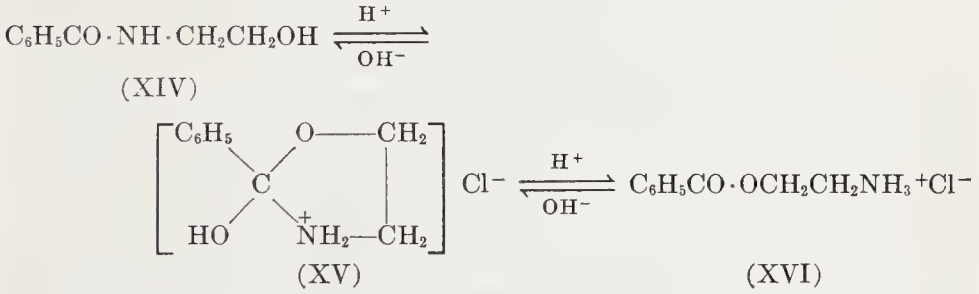
Finally, attempts to use the reactive side chain of aspartic or glutamic acid residues have been recorded and are discussed in Section IV-B-3.

The reader is invited to examine the various reactive side chains indicated in Table IV and speculate on the chances of exploiting their functional groups for the purpose of selectively cleaving the peptide chain to which they are attached.

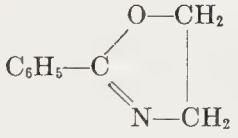
1. The N–O Acyl Migration

In 1924, Bergmann and Miekeley (28) demonstrated the migration of acyl groups from an *N*-acylamino alcohol to form an *O*-acyl analog under the influence of anhydrous acidic reagents. When treated with alkali the reaction was reversed.

As a simple example, let us consider the rearrangement of benzoic ethanolamide (XIV) to aminoethylbenzoate hydrochloride (XVI).



Phillips and Baltzy (238) have shown that this transformation proceeds via a labile intermediate hydroxyoxazolidine (XVI) compound, but a side reaction in the N—O direction involves an oxazoline intermediate formed by loss of water (XVII).

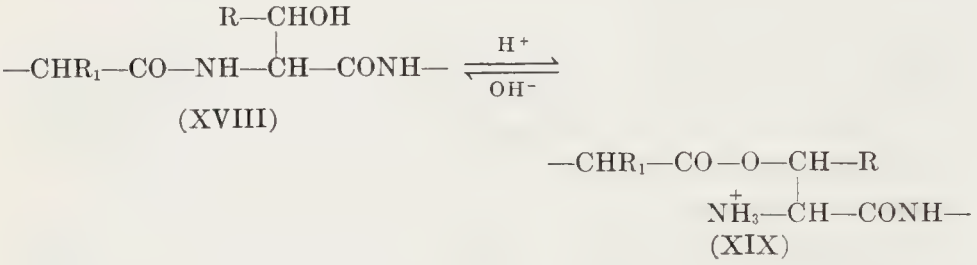


which in some cases may be isolated. Treatment with aqueous acid converts XVII into the ester (XVI).

The conditions for the N–O migration used by Phillips and Baltzy were 3.3*N* anhydrous ethanolic hydrogen chloride for 1 week at room temperature, when a 65% yield of aminoethylbenzoate hydrochloride was obtained. In contrast to the slow and nonquantitative reaction

in the N–O direction, the reverse reaction was instantaneous and substantially quantitative. Thus, within 2 min. of titrating compound XVI to pH 10 and back to pH 5, 95% of the ester had rearranged. An attempt to bring about the N–O transformation in dilute hydrochloric acid (0.04*N*) was unsuccessful.

In proteins, the same series of reactions is possible at the hydroxyl groups of serine and threonine residues, the normal peptide bond (XVIII) being converted to an *O*-peptidyl bond (XIX).



That this was the case during hydrolysis with concentrated acids became apparent (74,124) when analyses showed that the increase in hydroxyamino acid nitrogen (estimated by periodate oxidation or dinitrophenylation) was more rapid than that of amino nitrogen (Van Slyke method). This effect was more marked at low temperatures. Studies on the comparative rates of hydrolysis at 30° of *N*-benzoylalanine, *N*-benzoylserine, and *O*-benzoylserine with 10*N* hydrochloric acid (78) showed that in 4 days these compounds were hydrolyzed to the extent of 6, 27, and 58%, respectively. The greater stability of *N*-benzoylserine compared with *O*-benzoylserine shows that the acyl migration does not occur rapidly under these conditions, for otherwise the extent of hydrolysis would be similar. Further experiments (72) showed that the selectivity of the hydrolysis of edestin, ovalbumin, or hemoglobin was no greater in alcoholic hydrochloric acid, 10*N* sulfuric acid, or after pretreatment with thionyl chloride ($SOCl_2$) compared with 12*N* hydrochloric acid. Pretreatment with anhydrous sulfuric acid, however, followed by 6*N* hydrochloric acid at 18° for 6 hr. to hydrolyze the *O*-peptidyl bonds, increased the specificity of the cleavage. The best result obtained was with edestin, where 72% of the serine bonds and 33% of the threonine bonds were hydrolyzed 70 and 32 times, respectively, more rapidly than other peptide bonds.

The action of concentrated sulfuric acid on proteins converts aliphatic hydroxyl groups to monoesters of sulfuric acid, and some ring

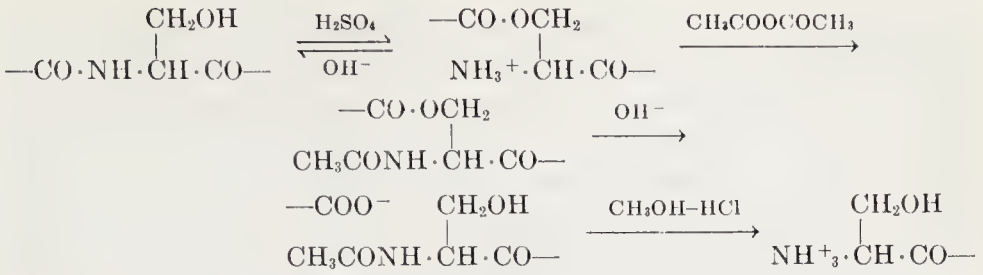


Fig. 13. Reactions used by Elliott (96) for selective cleavage of peptide chains at serine and threonine residues.

sulfonation occurs (252) during reaction times of 30 min. while warming from −35° to 20°. Insulin retained its biological activity (123) after sulfuric acid treatment. Prolonged treatment had been found to increase amino nitrogen values and give more dialyzable material on dilution with ice water. These results are those to be expected if an N–O acyl transformation occurred with subsequent hydrolysis of the peptide ester bonds in aqueous acid.

During reaction between sulfuric acid and reactive sites in proteins, water will be formed, and special precautions are necessary if it is desired to maintain anhydrous conditions. However, a comparison of concentrated and fuming sulfuric acid revealed no marked difference in the extent of rearrangement (72).

After 3 days at 21° in concentrated sulfuric acid, a maximum of 62% of the peptide bonds of silk fibroin (96,97) were converted to the *O*-peptidyl form. Although the reverse reaction may not be so rapid in proteins as in the case of aminoethylbenzoate discussed previously, because of changes in the folding of the peptide chains, it was found that exposure to pH 9 for several hours decreased the amino nitrogen considerably.

To render the cleavage of the ester bonds more specific than was the case with acid hydrolysis, Elliott acylated the exposed amino groups at pH 5. Treatment of the acylated protein with 0.01*N* alkali at room temperature for $1^1/_2$ hr. resulted in a large increase in the diffusible nitrogen, consistent with hydrolysis of ester bonds, to give a mixture of acetyl or formylseryl peptides. These were deacylated with cold methanolic hydrogen chloride; periodate oxidation of the product showed that free serine end groups accounted for 62% of the total number of series residues in the chain. These reactions are summarized in Figure 13.

In the case of silk fibroin, there are very few threonine residues compared with serine residues, and it was not surprising that no evidence of exposed threonine end groups was obtained. However, in other proteins studied since then which contain larger amounts of threonine, there is also a resistant fraction of serine residues, and threonine is much less reactive than serine (246,342). In lysozyme, for example, approximately 80% of the serine residues underwent the acyl shift compared with about 30% of the threonine residues (97).

Anhydrous sulfuric acid treatment of gluten (342) and gliadin (246) gave evidence of acyl migration involving serine residues (100 and 70%, respectively), but no evidence of migration involving threonine could be found (acetaldehyde formed on periodate oxidation). Both these proteins contain about one threonine residue for every three serine residues, so that the difference in reactivity is very striking.

Following Elliott (96), these treated proteins were acylated, saponified, and partially deacylated with methanolic hydrochloric acid. Attempts to fractionate the products by ionophoresis or chromatography were not successful; only single peaks were obtained. These difficulties may have been due in part to the introduction of sulfonic acid groups. With gliadin (246), an alternative method of blocking the free amino groups was tried. The exposed amino groups were destroyed by deamination with nitrous acid. This converted *N*-terminal serine residues to glyceric acid residues, and less serine was detected in hydrolyzates of the treated protein. Unfortunately, no experiments involving saponification of the deaminated protein, followed by attempts at fractionation, were reported, so that it is still not clear whether the expected peptide fragments could, in fact, be isolated. Desnuelle (71) has reported difficulty in dimethylating or deaminating amino groups (cf. 166) exposed by sulfuric acid in other proteins.

Ramachandran and McConnell (246) studied the amino nitrogen in relation to time of treatment with sulfuric acid. At $-35°$ or $0°$, the amino nitrogen values reached a maximum and then decreased, probably due to reaction of the amino groups to give sulfamates. This subsequent decrease of amino nitrogen makes the assessment of the optimum time of treatment impossible. Although Reitz *et al.* (252) found no evidence of reaction between sulfuric acid and amino groups, the reaction times in their studies were much shorter; i.e., approximately 30 min. compared with 2 or more days.

In general, however, amino nitrogen values are the most satisfactory measure of the extent of the acyl transformation. The amino groups in the rearranged proteins seem rather inaccessible to reagents. Thus, attempts (57,96) to couple the exposed amino groups with FDNB gave the expected DNP-serine (and DNP-threonine with lysozyme (97)), but the recoveries were much lower than would be expected on the basis of amino nitrogen values. Reaction under alkaline conditions introduces the complication of the reversible reaction, which, as we have discussed, is extremely rapid. At pH 5, the rate of reaction with FDNB is slower but the O–N transformation does not occur. At pH 8.5 at 20° for 2.5 hr., only 4.5% of the serine nitrogen of fibroin reacted with FDNB, whereas at pH 5 for 4 hr. at the same temperature, 23% reacted. The true figure was 62%, based on the decrease in amino nitrogen figures on standing at pH 9 for several hours (96).

In another study of rearranged lysozyme (166), no DNP-serine or DNP-threonine could be detected using FDNB at pH 5 for 8 hr. However, once the *O*-peptidyl bond is split, it is possible to get the expected results by periodate analysis for hydroxyamino acid end groups (97) or by dinitrophenylation (246).

It is perhaps surprising that the acetylation or formylation at pH 5 is so much better than dinitrophenylation. This conclusion relies on indirect evidence, since the acetylserine cannot be isolated as it is not stable to hydrolysis like DNP-serine. Moreover, the DNP-seryl protein could not be degraded in the same manner with dilute alkali as in the case of the acetylseryl protein, since the DNP groups were unstable (96). The discrepancy in the figures could be due to an increased destruction of the DNP-serine during hydrolysis, as was the case with other proteins (42,119).

Apart from the incomplete nature of the rearrangement, another drawback of the N–O acyl transformation as a preliminary step in the study of peptide structure is the report (246) that several of the constituent amino acids of gliadin were modified by sulfuric acid. These included amide groups, methionine, arginine, tyrosine, phenylalanine, and cystine.

Apart from a brief description of an ionophoretic fractionation of the fission products of sulfuric acid–treated lysozyme (97, p. 139), the only case where a peptide fraction obtained by this method has been characterized after isolation is with silk fibroin. After treatment with

chymotrypsin, a precipitate is formed representing 60% of the fibroin and composed predominantly of serine, glycine, and alanine residues with a small amount of tyrosine (81). This precipitate was treated with sulfuric acid at 27°, or preferably phosphoric acid (containing phosphorus pentoxide to insure that the acid remained anhydrous) at 40°, then cooled, diluted and coupled with FDNB at a pH that increased by stages to about 9. During this time, the ester bonds were hydrolyzed and the exposed amino groups dinitrophenylated. From the water-soluble DNP-peptides, a fraction identified as DNP-Ser.-Gly.Ala.Gly.Ala.Gly.OH was isolated, which accounted for 36% by weight of the chymotryptic precipitate and was considered a repeating unit in the molecule (206).

There has been considerable uncertainty and speculation regarding the possibility of the acyl transformation occurring in dilute aqueous media. Tanford (307) has studied the titration curves of several well-characterized proteins (insulin, ribonuclease, lysozyme, β-lactoglobulin, ovalbumin, and human serum albumin) and shown that for these, at least, there is no evidence of any increase in amino groups at pH 2, the lowest pH reached in these titrations. The evidence previously discussed suggests that the transformation occurs more readily in concentrated aqueous acids.

With anhydrous organic solvents containing acid, there is a possibility of particular hydroxyamino acid residues undergoing the acyl shift. Thus, in the Edman degradation (see Section V-B-1(a)) where the peptide derivative is treated with nitromethane–HCl (87), acetic acid–HCl (88), or dioxane–HCl (185) to cyclize the *N*-terminal residue, it has been found (2,313) that small amounts of *N*-terminal serine or threonine are detectable in subsequent stages, and in one case this has led to an erroneous sequence (2,185). Normally, the coupling with C_6H_5NCS or FDNB is performed at pH 8.5, and if the protein is exposed to this pH before the reagent is added, amino groups exposed by an acyl shift should revert to the *N*-peptidyl form. This would be a worth-while precaution in stepwise degradations where the acyl shift is a possible complicating factor.

Crawhall and Elliott (65) have studied the use of hydrogen chloride in anhydrous methanol or nitromethane to bring about the N–O acyl transformation in simple peptides The rearrangement was never quantitative and was accompanied by side reactions. Neither fuming sulfuric acid in nitromethane nor glacial acetic at 100° caused produc-

tion of amino nitrogen. Methanolic solutions of HCl or BF_3 were also found unsuitable by Bailey (14), but an 80% rearrangement of simple peptides was obtained with mixtures of BF_3 or $SnCl_4$ with formic or acetic acids for 1–4 hr. at 60–80°. Better yields (85–90%) were obtained by proceeding via the intermediate oxazolines (XVII) formed by phosphorus oxychloride at room temperature for 3 hr., and by subsequently opening the ring with water or dilute acid.

When a similar reagent, $POCl_2F$, was applied to insulin, 50% of the serine and threonine bonds underwent an N–O acyl transformation. With esterified insulin, the conversion was almost quantitative as udged by amino nitrogen values (13).

During esterification of proteins with 0.1*N* methanolic HCl, it has been found (56,57,215) that there is an increase in amino nitrogen that is reversed in alkali due to an N–O shift. In the case of insulin (56), the threonine residue was the more labile.

Another example of anhydrous acid conditions causing an N–O peptidyl shift has been the reversible inactivation of lysozyme in anhydrous formic acid at room temperature (166). In this case, the change was followed by estimating amino nitrogen values and by enzyme activity. Complete inactivation occurred in 16 hr. and, in addition to an increase in amino nitrogen values, the protein acquired an increased net positive charge reflected in its electrophoretic mobility. These changes were completely reversed in aqueous solutions at pH 8.5. At pH 7.5, the reverse change was incomplete, as judged by electrophoresis and alkali consumption, although full enzyme activity was restored. The number of peptide bonds undergoing the N–O peptidyl shift could not be established with certainty, but a minimum of eleven of the nine serine and seven threonine residues (311) were involved.

2. Bromine Water

In degradative work on oxytocin (84,220,254), oxidation of the peptide was attempted with bromine water. In contrast to the single product obtained with performic acid oxidation, two peptide fragments were obtained using bromine water. Treatment of the performic acid–oxidized peptide with bromine water also gave the same two fragments, and the degradation is illustrated in Figure 14.

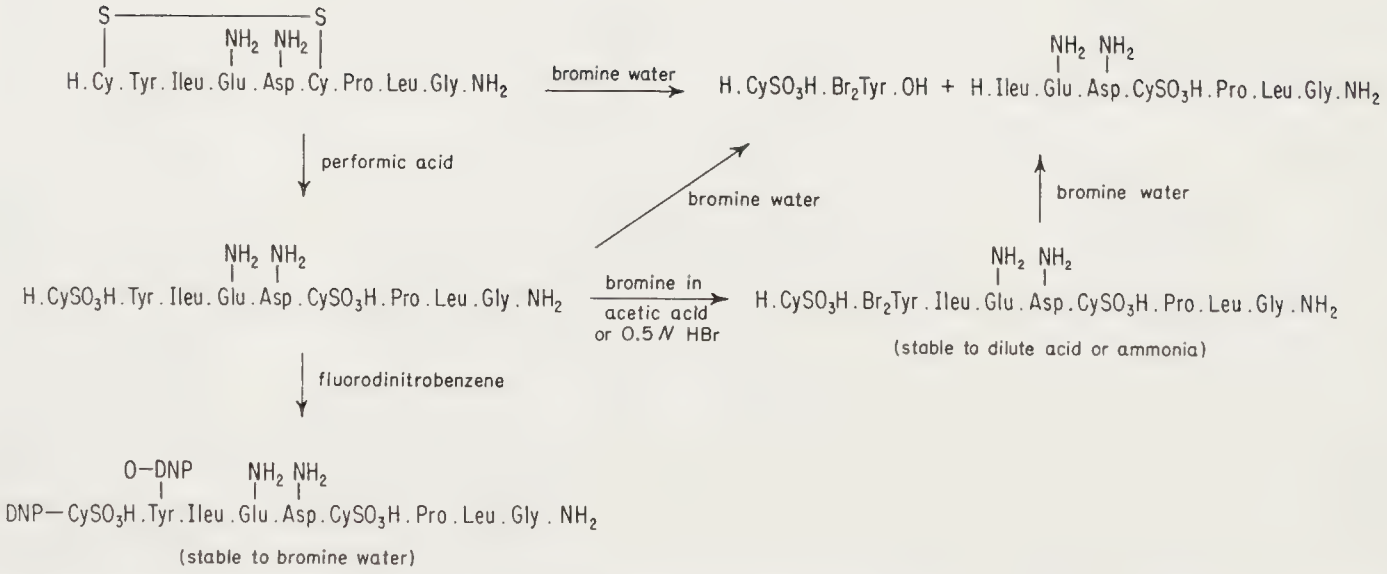


Fig. 14. The selective cleavage of oxytocin derivatives by treatment with bromine water. Br_2Tyr represents a 3,5-dibromotyrosyl residue.

Thus, oxidation of the half-cystine residues to β-sulfoalanyl residues, bromination of the tyrosine, and cleavage of a tyrosylisoleucyl bond have taken place. The reaction does not depend on the isoleucyl residue, since in vasopressin, where phenylalanine replaces the isoleucine, a similar cleavage with bromine water occurs (241). In the case of vasopressin, considerable decomposition took place during the fragmentation, and only the large fragment was isolated.

Bromine water contains approximately 3.5% bromine and the pH of the solution is about 2. In further studies of this unusual cleavage (253), it was reported that bromination of the tyrosine in performic acid–oxidized oxytocin could be effected without simultaneous cleavage, both in glacial acetic acid solution and in aqueous solution, provided the solvent was 0.5*N* with respect to HBr. Subsequent treatment of this brominated, oxidized oxytocin with 0.05% acetic acid at room temperature for 24 hr., or with 1*N* HBr or dilute NH_3 at -5 to $-10°$ for 45 min., did not cause cleavage of the molecule. If, however, the product was treated with bromine water at -5 to $-10°$, then the two peptide fragments were produced. The cleavage is related to the susceptibility of the tyrosine ring to halogenation, as was shown by the fact that the DNP-oxidized oxytocin, in which the phenolic group is substituted with a dinitrophenyl group, underwent neither cleavage nor substitution of the benzene ring when treated with bromine water.

This fragmentation is one of the few known selective degradations by a chemical method, but there is not sufficient data to formulate a theory for the reaction. Perhaps the proximity of the brominated tyrosyl residue and the β-sulfoalanyl residue labilized the bond involving the tyrosyl carboxyl.

It would be interesting to study the effect of bromine water on oxidized ribonuclease where both -$\underset{96}{\text{Cy}}\text{SO}_3\text{H}.\underset{97}{\text{Tyr}}.\underset{98}{\text{Lys}}$- and -$\underset{25}{\text{Tyr}}$.-$\underset{26}{\text{Cy}}\text{SO}_3\text{H}.\underset{27}{\text{Asp}}(\text{NH}_2)$- sequences are present. In oxidized insulin, there is a -$\underset{18}{\text{Asp}}(\text{NH}_2).\underset{19}{\text{Tyr}}.\underset{20}{\text{Cy}}\text{SO}_3\text{H}.\underset{21}{\text{Asp}}(\text{NH}_2)$.OH sequence in the A chain, and both oxidized insulin and the separated A chain, on treatment with bromine water at -5 to $-10°$, have given rise to the peptide $\text{Cy}\text{SO}_3\text{H}.\text{Asp}(\text{NH}_2)$, indicating splitting of the bond following the tyrosine residue (318). The quantitative aspects of this splitting have yet to be investigated.

3. Selective Cleavage Involving Aspartyl or Glutamyl Residues

(*a*) *The Preferential Release of Aspartic Acid from Proteins.* The peptide bonds on either side of the aspartic acid residue in a protein molecule are peculiarly susceptible to dilute acid hydrolysis (232). This effect is a function of the pH of the solution and not of the strength of the acid concerned (32,188). Thus, bovine serum albumin released 44% of the aspartic acid residues as the amino acid after 18

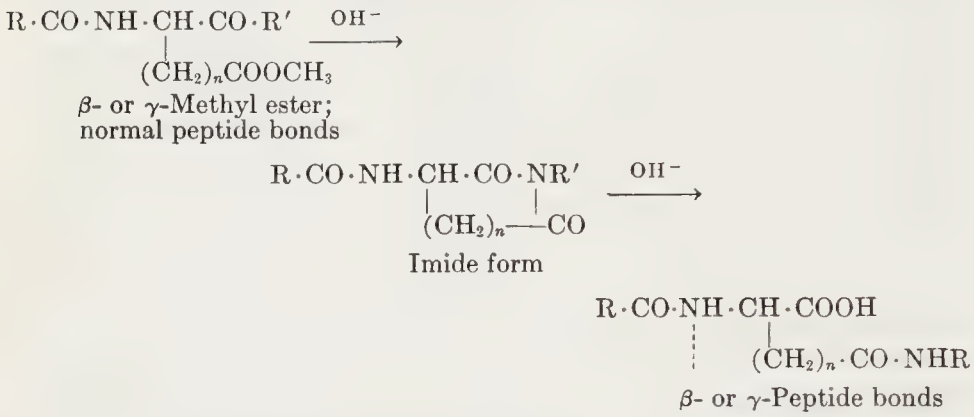


Fig. 15. The alkaline rearrangement of the β- or γ-esters of aspartyl or glutamyl residues. The bond indicated by the dotted line is similar to a *C*-terminal bond and should be cleaved by reagents selective for *C*-terminal residues.

hr. at 100° and pH 2.14, whereas only 26% was liberated at pH 3.15 (188). When elastin was extracted with 0.25*M* oxalic acid at 100°, a soluble protein was obtained, and aspartic acid was the only free amino acid released (234). However, the presence of small peptides and end-group analysis (24,233) showed that a considerable degree of hydrolysis of other peptide links had occurred. From studies with model compounds (73), it was concluded that serine and threonine bonds would be labile. Application of this method of hydrolysis to the A chain of oxidized insulin (267) showed that bonds involving cysteic acid residues and the amino groups of serine and threonine residues were also broken. The release of aspartic acid was not quantitative, and low yields of a large variety of peptides were obtained.

Schroeder (277) has also reported low yields of peptides under these conditions. The specificity of the method is therefore not as great as the release of free amino acids would suggest, and it is clearly unsuitable for application in the selective degradation of a protein.

(*b*) *The Rearrangement of Aspartyl and Glutamyl Peptides.* By treatment with dilute alkali, the β- or γ-esters of acylaspartyl or acylglutamyl peptides are rearranged via an intermediate imide to form an acyl peptide with β-aspartyl or γ-glutamyl linkages (21). Since these isomers then contain an α-carboxyl group similar to a *C*-terminal residue, it was envisaged that application of one of the methods developed for stepwise removal of a *C*-terminal residue would result in selective fission at the bonds involving the amino groups of the rearranged aspartyl or glutamyl residues. The course of this reaction is illustrated in Figure 15.

Model compounds such as the ethyl ester of *N*-benzoyl-α-aspartylglycine-*n*-hexylamide, when treated at 40° with 0.28*N* Na_2CO_3 for 4 hr., gave 75% of the β-aspartyl peptide and 25% of the normal peptide (de-esterified starting material). The rate-limiting step with aspartyl compounds was the hydrolysis of the imide, which with short reaction times could be isolated. The corresponding asparaginyl derivative only reacted to a small extent.

In the glutamic acid series, the reaction requires more vigorous conditions. Thus, the ethyl ester of *N*-benzoyl-α-glutamylglycine-*n*-hexylamide was comparatively stable to Na_2CO_3 at 50°, 64% being unchanged after 7 hr. However, 0.1*N* NaOH at room temperature for 4 hr. gave 57% of the γ-glutamyl peptide and 43% of the α-glutamyl peptide. In the glutamic acid series, the rate-limiting step is the ring closure of the ester to the imide, in contrast to the case in the aspartic acid series.

The second step, cleavage of the peptide bond, has not yet been described for these model compounds. However, since the selective *C*-terminal methods do not give high yields on simple compounds, it may be concluded that the over-all yield for the selective splitting of peptide bonds would be small and the peptide mixture obtained rather complex.

(*c*) *Condensation of the γ-Carboxyl Group of α-Glutamyl Peptides with the Peptide Chain.* This reaction has been investigated by two groups of workers (22,62). By reaction of esters of *N*-acylglutamic acids with thionyl chloride at 0°, high yields of the corresponding 1-acylpyrrolid-2-ones are obtained. This reaction is illustrated in Figure 16 for *N*-benzoyl-α-glutamylglycine-*n*-hexylamide (22). Treatment with dilute alkali gives a selective cleavage of the chain at the peptide bond involving the amino group of the glutamyl residue,

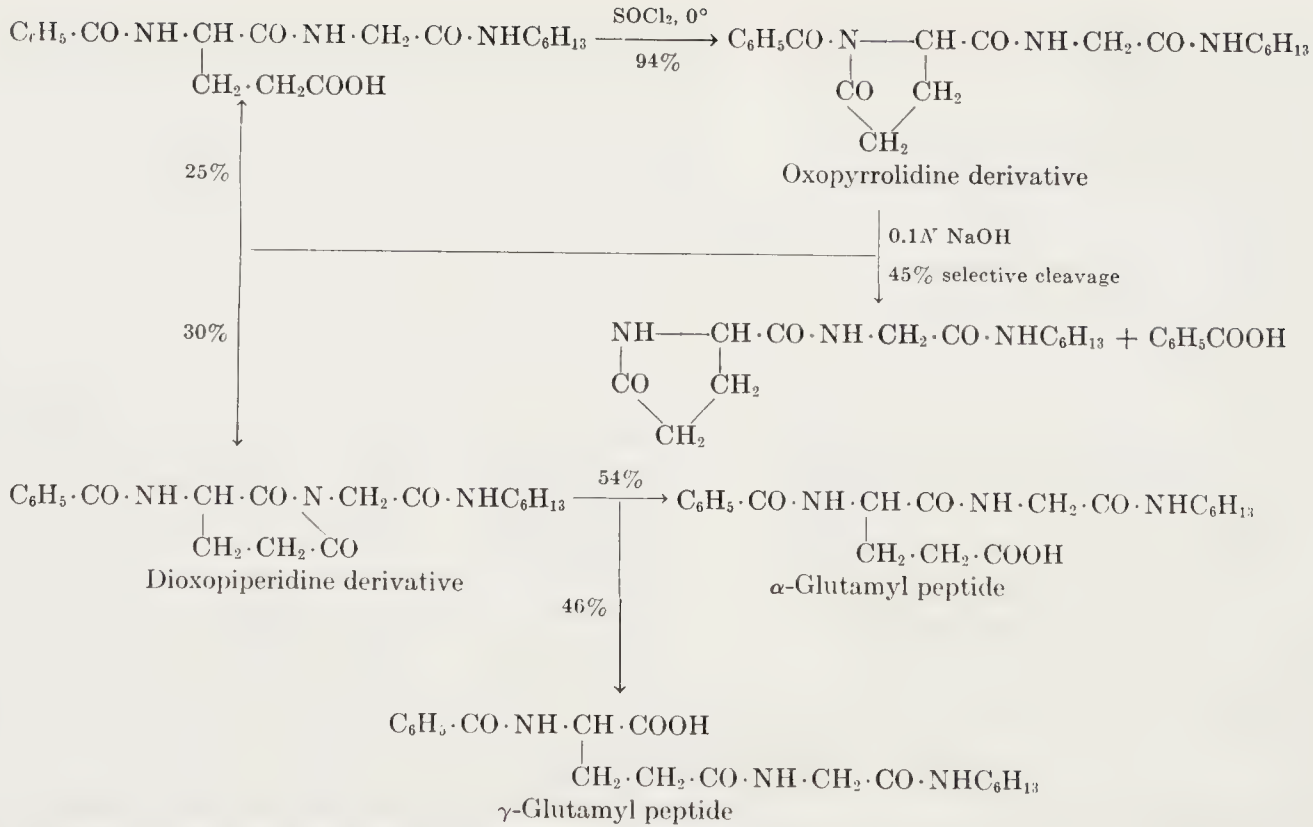


Fig. 16. Cyclization of the γ-carboxyl groups of *N*-benzoyl-α-glutamylglycylhexylamide and the action of dilute alkali on the product.

but competing reactions to re-form the original structure and the γ-glutamyl analog considerably reduce the yield. With *p*-toluenesulfonylglycylglycyl-α-glutamylphenylalanylcyclohexylamide, on the other hand, although an excellent yield of the cyclized oxopyrrolidine derivative was obtained, alkaline hydrolysis only regenerated the original α-compound (90%) (62).

Under certain cyclization conditions, the six-membered ring imide, a dioxopiperidine derivative (Fig. 16), may be formed, and this compound on alkaline treatment may give the γ-glutamyl isomer of the original glutamyl peptide as the main product.

Although a certain amount of fission of a peptide chain may be achieved by this type of reaction under comparatively mild conditions, it is obvious that the course of the reaction is controlled to a large extent by the particular sequence involved. The rearrangement to γ-glutamyl isomers and the relatively poor yields obtained suggest that, in its present stage of development, the method is unsuitable for selective fission of long peptide chains.

V. Reagents Selective for Terminal Residues

A. ENZYMES

Enzymic methods are available for the identification of both *N*- and *C*-terminal residues. The advantages of these processes are that reaction occurs under mild experimental conditions, and only small amounts of material are required, since the liberated amino acids may be detected in micro quantities by paper chromatography (114,319). Sequences can be deduced in suitable cases since the liberation of the original *N*- or *C*-terminal residue exposes a new terminal group, which may also be liberated by the enzyme, although the rate may vary from very slow to as fast as that of the first amino acid.

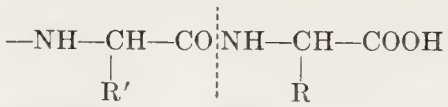
Since no racemization or denaturation is involved in this enzymic removal of terminal groups, the products from biologically active substrates are suitable for examination for the effect of loss of these residues on biological activity (10).

Disadvantages of these methods are their limitation to amino acid residues of the L-configuration and with free α-amino or α-carboxyl groups; moreover, bonds involving certain residues may not be attacked by the enzymes. In addition, the influence of preceding residues may be sufficient to prevent hydrolysis of some residues

which specificity studies would suggest were suitable for liberation. A most important requirement of the enzymes discussed is their freedom from contaminating endopeptidases. Small quantities of these impurities could result in internal peptide bond splitting to form additional substrates for the enzyme. Similarly, the purity of the substrate must be such that impurities cannot complicate the interpretation of the results.

1. Carboxypeptidase

This enzyme may be isolated from pancreatic extracts in a pure condition (128), but before use for sequence studies, it is preferably incubated with diisopropyl fluorophosphate to inhibit any traces of chymotrypsin, trypsin, or subtilisin (122,266,299). The general properties and specificity of carboxypeptidase have been discussed in detail elsewhere (227,292), and the application of the enzyme in sequence studies on proteins has also been reviewed (114,136,225,319). The enzyme is specific for *C*-terminal amino acids in peptides or proteins which possess a free α-carboxyl group of the general structure



C-terminal amide residues as in oxytocin or vasopressin (85) are resistant.

The rate of liberation of *C*-terminal residues is governed mainly by the nature of its side chain, R, but the neighboring side chain, R′, may also influence the rate of reaction. Aromatic amino acids are the most rapidly liberated (hence it is particularly necessary for chymotrypsin to be absent), followed by terminal residues with larger aliphatic side chains. As the size of nonpolar side chains is decreased or as the group becomes more polar, the rate of cleavage is retarded.

Thus, glycine and glutamic and aspartic acids are liberated slowly, and arginine and lysine are liberated at a rate dependent on the purity of the enzyme. More recently, a new basic carboxypeptidase present in less purified carboxypeptidase preparations has been described (104,105) which attacks *C*-terminal peptide bonds involving the amino groups of lysine and arginine specifically.

Proline and hydroxyproline are completely resistant to the enzyme. Cysteine has not been detected as a reaction product, and half-

cystine, if present, may be released from peptide linkage but remains attached to the polypeptide chain by the disulfide bond. Oxidation of cystine residues to cysteic acid will not give a residue suitable for liberation by carboxypeptidase due to the charged side chain, but reduction and alkylation to —SCH_2CONH_2 groups gives a neutral residue that has recently been detected (197) in carboxypeptidase digests of reduced and alkylated prolactin, indicating that a *C*-terminal half-cystine residue is present.

The influence of the preceding amino acid residue on the rate of liberation of the terminal group is best illustrated by the effect of prolyl residues. Thus, in insulin with a *C*-terminal -Pro.Lys.Ala.OH sequence in the B chain, the alanine is rapidly liberated, while the combination of the polar side chain of the lysyl residue and the penultimate prolyl residue inhibits release of lysine (136,266). In tobacco mosaic virus with the *C*-terminal sequence -Pro.Ala.Thr.OH (37, 228), only threonine is liberated (138), whereas in corticotropin, with the *C*-terminal sequence -Pro.Leu.Glu.Phe.OH, the rate of release falls in the order phenylalanine, glutamic acid, leucine (136,283,338), reaction ceasing only when proline becomes *C*-terminal.

The determination of the number of peptide chains in a protein by a quantitative study of the rate of release of amino acids may be equivocal because two adjoining residues may be liberated at almost identical rates. This occurred in the case of bovine growth hormone, where two residues of phenylalanine were rapidly released by carboxypeptidase, followed by alanine, leucine, and serine. Although two *N*-terminal residues are present in this protein, there is only one phenylalanine residue detectable as *C*-terminal by hydrazinolysis. The carboxypeptidase results are due to a *C*-terminal -Phe.Phe.OH sequence (197).

The interpretation of the data for a protein is, in general, less complicated when it is known with some degree of certainty that a single open peptide chain is present. If the amino acid residues are arranged in order of decreasing rates of hydrolysis starting from the *C*-terminal residue, then valid deductions of the sequence are often possible. However, the difficulties are illustrated in the case of ribonuclease, a single peptide chain, where a *C*-terminal -Asp.Ala.-Ser.Val.OH sequence has been established (248). The original experiments (11) with carboxypeptidase suggested a Met.Tyr.Ala.(Leu or Ileu,Phe).Val.OH sequence, and it was only with a carboxypeptidase

preparation more thoroughly treated with diisopropylfluorophosphate that evidence for a -(Ala,Ser).Val.OH sequence was obtained (248).

It should be pointed out that in the case of ovalbumin (229), prolactin (197), yeast triosephosphate dehydrogenase (132), chymotrypsinogen, trypsin, and trypsinogen (224), oxytocin and vasopressin (85), α-melanophore-stimulating hormone (139), and carboxypeptidase (319), *C*-terminal residues are present but are unreactive to the enzyme.

In α-lactalbumin and β-lactoglobulin (225), insulin (266), tobacco mosaic virus (138), and lysozyme (165,317), the results are remarkably clear cut in that only *C*-terminal residues are liberated, the penultimate residues forming part of a peptide bond that is resistant, owing to the nature of its side chain or adjacent residue.

Carboxypeptidase is not inactivated by 6*M* urea at pH 8. Although the rate of liberation of phenylalanine from carbobenzoxyglycylphenylalanine was only 25–50% of the rate in the absence of urea, these conditions gave rapid liberation of amino acids from yeast triosephosphate dehydrogenase (132). In the absence of urea, this substrate was not digested by the enzyme.

2. Leucine Aminopeptidase

This enzyme is widely distributed in mammalian tissues in very small amounts. Recently (149,298), highly purified samples of leucine aminopeptidase have become available, following the discovery that Mg^{2+} greatly increases its stability. Maximum activity is obtained in the presence of Mn^{2+} at pH 9.1–9.3, but the enzyme is rapidly inactivated above pH 9, and, for experiments extending over several hours, a pH of 8.0–8.5 is used at 40° with Mg^{2+} or Mn^{2+} as activator.

Leucine aminopeptidase attacks *N*-terminal peptide bonds in peptide chains in a manner similar to the action of carboxypeptidase on *C*-terminal residues. The purified enzyme has no endopeptidase activity and, since it is not affected by diisopropyl fluorophosphate, a preliminary incubation with this inhibitor serves to inhibit possible proteinase impurities.

The action of the enzyme on synthetic substrates has been the subject of several comprehensive reviews (292,294,296). Its substrates are not limited to leucine compounds, as its name suggests. The rate of hydrolysis of various compounds is predominantly a function of the nature of the residue bearing the free amino group. In general,

the rate of hydrolysis of the straight-chain aliphatic compounds is greatest for those with largest side chain—e.g., leucine amide—and decreases with the size of the grouping. Aromatic compounds are less sensitive than the larger aliphatic ones, whereas compounds with polar groups in the side chain of the residue bearing a free amino group are hydrolyzed relatively slowly.

Leucine aminopeptidase has a much higher turnover number—i.e., moles of substrate decomposed per minute per unit weight of enzyme —than carboxypeptidase, which is considerably more active than papain, one of the most active proteinases (296). Because of its high activity, even the less sensitive bonds in peptide chains may be hydrolyzed at an appreciable rate. Moreover, the specificity is not limited by certain types of residues, as is the case with carboxypeptidase. Thus, half-cystine residues are freed from peptide linkage; proline and the amino acids with polar side chains are also liberated, although, of course, the rates may be slow. Although advantageous for some purposes, this broader spectrum of action increases the difficulties of deducing valid sequences from a study of the rate of liberation of amino acids (149) from peptide chains.

With long-chain substrates (149), it has been found that some native structures are resistant to the enzyme, whereas the unfolded, oxidized, or denatured protein is readily digested. Thus, zinc insulin is scarcely attacked, but removal of the zinc facilitates hydrolysis in a stepwise manner. The hydrolysis in this case proceeded past the disulfide bridges. The separate A and B chains of oxidized insulin were readily hydrolyzed. Amino acid analyses indicated that every bond in the A chain was hydrolyzed, whereas in the B chain the slower action permitted the determination of the sequence of the first six residues in the chain.

Whereas native β-lactoglobulin and growth hormone are slowly hydrolyzed by leucine aminopeptidase, native human and bovine plasma albumins, lysozyme, and ribonuclease were resistant. However, preliminary oxidation of human plasma albumin, lysozyme, or ribonuclease with performic acid resulted in digestion of these substrates and the liberation of amino acids in accord with their known N-terminal sequences. Denaturation of human albumin with $8M$ guanidine hydrochloride for 24 hr. at pH 8.5 did not increase its digestibility relative to the native protein.

Leucine aminopeptidase hydrolyzed oxytocin rather slowly, prob-

ably because of the *N*-terminal half-cystine residue, since hypertensin (98) (Fig. 10) of similar size was rapidly broken down to amino acids. Glucagon (Fig. 4), an open-chain polypeptide, was also digested completely to free amino acids (149).

Complete digestion by this means establishes the L-configuration for all amino acids in the molecule, since the enzyme does not liberate D-amino acid residues.

The most extensive degradation of a protein chain by leucine aminopeptidase has been in the case of mercuripapain. With low enzyme concentrations, the first nineteen residues are liberated in essentially stoichiometric amounts in 24 hr. Reaction apparently ceased when an arginine residue became *N*-terminal. With higher enzyme/substrate ratios, a more extensive degradation occurred until two-thirds of the protein was liberated as amino acids (148). The residual protein on activation still possessed full activity on a molar basis toward benzoylarginineamide, showing that the active center must be in the *C*-terminal portion.

Data on the amino acids released by extensive breakdown of mercuripapain are still unavailable, but no proline is present in the first nineteen residues. The occurrence of free proline in digests is of interest following the experiences of Li and co-workers (121) with leucine aminopeptidase on substrates of known structure containing proline. They found that when bovine or porcine MSH (Fig. 9) was incubated with the enzyme, more aspartic acid (1.0 mole) was released from the bovine material with sequence H.Asp.Glu.Gly.Pro- than from porcine MSH (0.43 mole) with sequence H.Asp.Ser.Gly.-Pro-. Glycine was liberated only in small amounts from bovine MSH and not at all from the porcine material. This is an illustration of the effect of different adjacent amino acids on the rate of release of identical *N*-terminal residues. In both cases, low yields of glycine were attributed to adjacent prolyl residues. In support of this thesis, which does not accord with the complete digestion of hypertensin (Fig. 10), only valine was liberated from a peptide with *N*-terminal sequence H.Val.Tyr.Pro- in 24 hr. However, removal of the valine and tyrosine by the Edman method gave an *N*-terminal prolyl group which was liberated by the enzyme. *N*-terminal proline in protamines was also liberated (7). Performic acid oxidized prolactin with *N*-terminal sequence H.Thr.Pro.Val- also gave no liberation of amino acids.

In connection with the increased digestibility of unfolded protein

molecules, it is of interest that leucine aminopeptidase retains some activity in 8*M* urea or 6*M* acetamide solutions and that the inactivation which occurs is fully reversible. In guanidine hydrochloride solutions, inactivation is rapid and irreversible.

B. CHEMICAL

The methods discussed in this section have been limited to those which are capable of application in a stepwise fashion, so that, apart from the removal of the terminal residue, the peptide chain remains intact. This excludes from discussion the DNP method of Sanger (261), which has given the most information regarding the *N*-terminal amino acids of peptides and protein. However, numerous comprehensive reviews are available (114,276,319). Similarly excluded is the hydrazinolysis method of Akabori and co-workers (3,4,229) for *C*-terminal residues, which has been improved (39) so that it is now capable of identifying all the common amino acids.

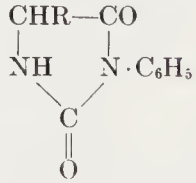
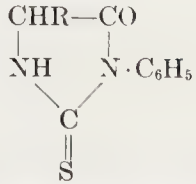
It is, of course, an essential requirement with a stepwise procedure that high yields be obtained at each step, and that the reagents should not split peptide bonds. From the discussion of the most successful of these methods, that of Edman, it will be apparent that the best reaction conditions are not yet definitely established. The possibility of peptide bond splitting makes it more suitable for peptides isolated from protein hydrolyzates than for the proteins themselves. In any case, a preliminary fragmentation of a protein and identification of the fragments is essential for the accumulation of sufficient data to allow deduction of the complete amino acid sequence of the protein.

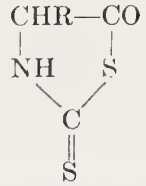
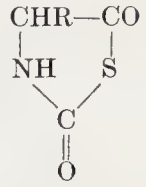
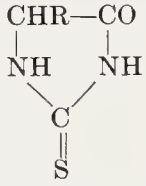
There have been numerous other methods proposed for the peptides from time to time which cannot be adequately treated here, but which nevertheless involve the exploitation of subtle chemical reactions. In general, these methods (106,319) either have given only poor yields, or have not been examined sufficiently to assure their usefulness with the wide variety of reactive side chains that are present in natural peptides or proteins.

1. Methods for N-Terminal Residues

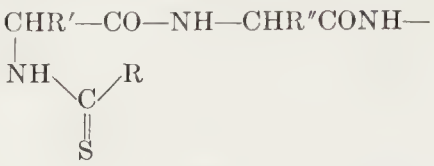
(*a*) *Use of Phenyl Isothiocyanate and Related Methods.* There have been several reagents proposed for the selective cleavage of the *N*-

TABLE V
Reactions for Stepwise Degradation of Peptides from α-Amino Group

Reagent	Condensation product	Cyclized derivative	Ref.
Phenyl isocyanate, $C_6H_5 \cdot NCO$	$C_6H_5 \cdot NH \cdot CO \cdot NH \cdot CHR \cdot CO—$	 5-substituted-3-phenylhydantoin	1
Phenyl isothiocyanate, $C_6H_5 \cdot NCS$	$C_6H_5 \cdot NH \cdot CS \cdot NH \cdot CHR \cdot CO—$	 5-substituted-3-phenylthiohydantoin	87

Carbon disulfide, CS_2	$^{-}S \cdot CS \cdot NH \cdot CHR \cdot CO—$	 4-substituted-2-thiothiazolid-5-one	190
Methyl methoxy dithiocarbamate (Dimethyl xanthate), $CH_3O \cdot CS \cdot SCH_3$	$CH_3O \cdot CS \cdot NH \cdot CHR \cdot CO—$	 4-substituted-thiazolid-2,5-dione	175
Methyl *N*-acetyl dithiocarbamate, $CH_3 \cdot CO \cdot NH \cdot CS \cdot SCH_3$	$CH_3CO \cdot NH \cdot CS \cdot NH \cdot CHR \cdot CO—$	 5-substituted-2-thiohydantoin	99

terminal residue from a peptide chain, based on the formation of an *N*-substituted derivative of type XX.



(XX)

Treatment of these derivatives with acid results in a selective cleavage of the first peptide bond and formation of cyclic thiohydantoins or related compounds. These cyclic compounds may be extracted into organic solvents and identified by chromatographic methods or regenerated to the amino acid.

The different reagents are shown in Table V. They all react under mild conditions (pH 8.5, low temperatures) to give derivatives which may vary in their rates of cyclization and their solubilities in the acidic cyclization reagents. Although they have all been applied successfully to simple peptides, only the phenyl isothiocyanate method of Edman (Fig. 17) has been used extensively on natural products. It has given information on *N*-terminal sequences in proteins, as well as of peptide fragments. The following discussion will therefore be limited to the methods based on Edman's phenyl isothiocyanate reagent.

Several substituted isothiocyanates, 3,5-dinitro-4-dimethylaminophenyl isothiocyanate (251), or *p*-azophenylphenyl isothiocyanate (247), have been proposed instead of phenyl isothiocyanate because they form colored derivatives. *p*-Iodophenyl isothiocyanate (50) has also been suggested because of the possibilities of radioactive labeling. These reagents have not yet received wide application, nor is it clear from the data given by the authors whether derivatives of the reagent cyclize more readily than those of the unsubstituted parent molecule.

The coupling of phenyl isothiocyanate with amino or α-imino groups proceeds smoothly at pH 8–9 and is usually performed in pyridine–water or dioxane–water at 40°. The desired pH is maintained by addition of small amounts of sodium hydroxide or trimethylamine. By measuring the alkali consumption as a function of time, the end point of the reaction is readily determined and information gathered regarding the number of reacting groups (230).

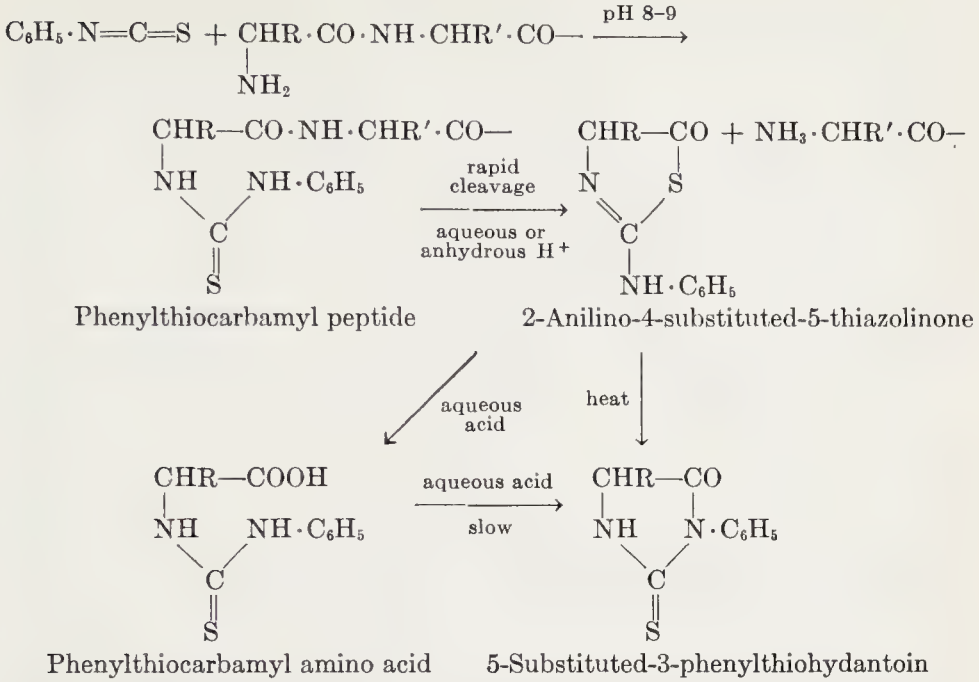


Fig. 17. Reactions involved in the phenyl isothiocyanate stepwise degradation of peptides, according to Edman (89).

The cyclization of the phenylthiocarbamyl (PTC) derivatives has been effected under a variety of acidic conditions. Difficulties are encountered as one proceeds from amino acids to peptides of increasing size.

The cyclization of the derivatives of amino acids is usually more difficult, particularly with glycine, than the cyclization of these same amino acids when in peptide combination. For the preparation of the phenylthiohydantoin (PTH) (86,91) derivatives, or for the quantitative estimation of *N*-terminal residues, the PTC compounds are often cyclized by 1*N* HCl at 100° for 1 hr. However, under these conditions, the PTH's of serine, threonine, and cystine are unstable and hence cannot be isolated or estimated. Moreover, all PTH's are subject to decomposition under acid conditions, the extent of destruction increasing with the strength of the acid and the temperature (114,316). In aqueous solution, the maximum yield of PTH's is obtained using strong acid at relatively low temperatures for the shortest possible time. By using low temperature and concentrated acids (1–5*N*), the PTH's of serine, threonine, and cystine have been

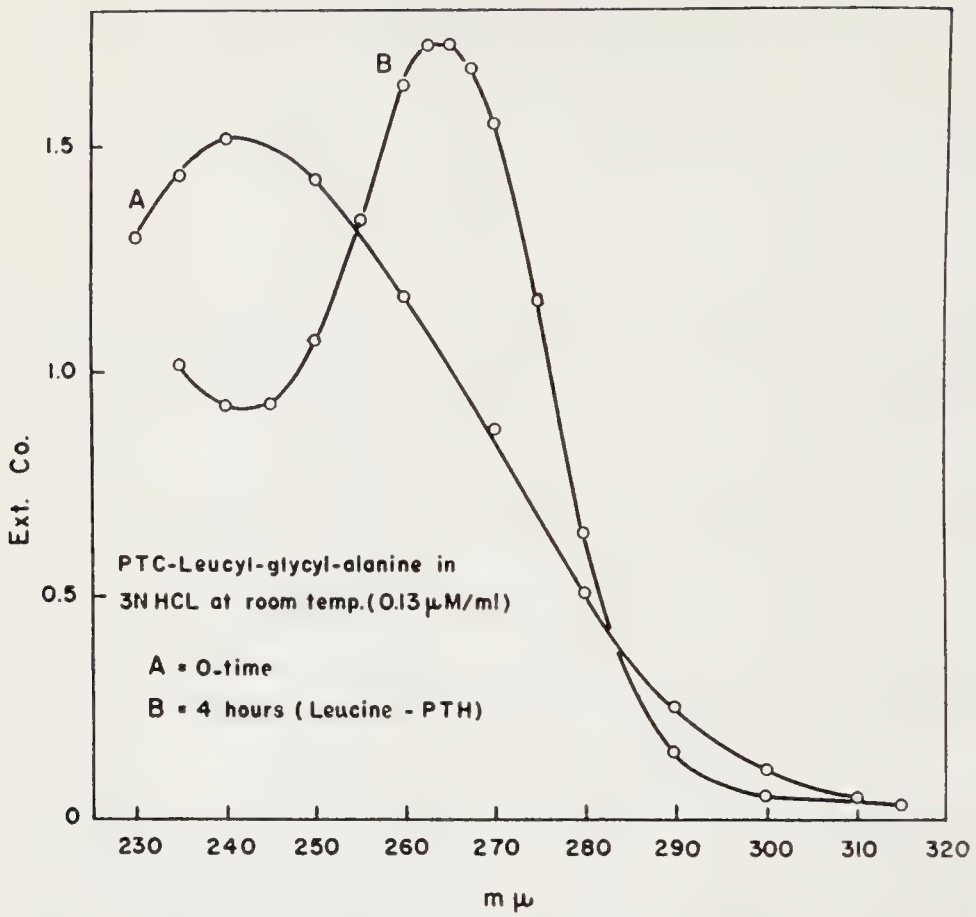


Fig. 18. Ultraviolet absorption curves of PTC-leucylglycylalanine (A) and leucyl-PTH (B) formed from it in 3*N* acid (from ref. 114).

prepared (158,194) in aqueous solution. They are also readily prepared in acetic acid–HCl (288).

For the stepwise degradation of peptides, a method has been described (113,114) in which the PTC-peptide is dissolved in 3*N* HCl at room temperature and the completion of the cyclization determined from the change in absorption over the range 235–275 mμ. As the PTH of the *N*-terminal amino acid is released, the absorption shifts from a maximum at about 240 mμ to a maximum at 262.5 mμ, with a minimum near 240 mμ (Fig. 18). If the reaction proceeds slowly, it is carried out at a higher temperature, e.g., 40°. Usually, reaction times at room temperature are 1.5–24 hr. When cyclization is complete, the PTH is extracted into ethyl acetate and the aqueous solu-

tion evaporated to dryness at low temperature to avoid unnecessary exposure to the acid and the possibility of peptide bond hydrolysis.

The yield of PTH can be calculated from the maximum optical density of the acid solution (if no aromatic amino acids are present) and also from the maximum optical density of the extracted PTH in ethanol (absorption maximum usually 267.5 mμ) using the extinction coefficients of the identified PTH (114,194,288). The PTH derivatives of arginine and histidine cannot be extracted from acid solution, but PTH-histidine can be extracted from neutral solution. The presence of PTH-arginine is detected from its absorption maximum (268 mμ) in the neutral aqueous solution and by paper chromatography.

This method has been applied with limited success to several soluble long-chain peptides such as oxytocin, the A chain of oxidized insulin (113), and hypertensin (98). Difficulties were encountered at the second half-cystine residue after five treatments of oxytocin, and at β-sulfoalanyl residues, corresponding to cysteic acid, after five treatments of the A chain. With hypertensin, which contains no cystine, the procedure was only successful for the first four residues, after which the yields were low. The absorption of aromatic amino acids or artifacts interfered with observation of the course of cyclization by periodic measurements of the absorption.

The PTH's may be identified by paper (92,185,287) or partition column (289) chromatography. The latter method is suitable for quantitative estimations, but rapid semiquantitative determinations are also possible after eluting from paper chromatograms. The spots are detected by an iodine-azide reagent specific for divalent sulfur or, for quantitative work, by the high ultraviolet absorption of the phenylthiohydantoins (92).

For regeneration of amino acids from the thiohydantoins, either barium hydroxide (86) at 140° for 48 hr. (or 2 hr. (283)) or 6*N* HCl at 150° for 16 hr. (193) has been widely used. Others (98,274) have preferred hydrogen iodide at 140°. Direct identification of PTH's is preferable, since tryptophan, arginine, serine, threonine, cysteine, and cystine are destroyed or only partly regenerated by either acid or alkali with the formation of other ninhydrin positive substances. The yield of other amino acids is quantitative by either acid (193; but cf. 108) or alkaline (283) hydrolysis.

In the original procedure of Edman (87), aqueous acid was avoided

so that random splitting of peptide bonds did not occur. Anhydrous reagents such as nitromethane, dioxane, or glacial acetic acid (88) solutions of hydrogen chloride were used and found to give practically instantaneous cleavage of PTC-peptides at room temperature. Applied to higher PTC-peptides and to PTC-proteins, these reagents gave poor yields of PTH's (114,283,319), possibly because of the insolubility of the PTC derivatives or reaction conditions that were too severe.

The striking difference between the rate of cleavage of a PTC-peptide in aqueous or anhydrous acids prompted Edman to examine the mechanism of the cleavage reaction (89).

The estimation of the rate of reaction in anhydrous media had been based on amino nitrogen values and not on the yield of PTH. Paper chromatographic examination showed that the compound formed rapidly from PTC-leucylglycine was not leucinephenylthiohydantoin but 2-anilino-4-isobutyl-5-thiazolinone (Fig. 17) with an absorption maximum at 252 mμ. Heating (e.g., recrystallization) was sufficient to convert this into leucine-PTH. On the other hand, this compound was converted directly in anhydrous acid into leucine-PTH, whereas in aqueous media the conversion proceeded via the PTC-leucine and was much slower. Thus, there is little difference in the rates of cleavage in aqueous and nonaqueous media but a marked difference in the rates of cyclization to the PTH's in the two systems.

For stepwise degradation of higher PTC-peptides or PTC-proteins, anhydrous acidic reagents seem preferable in view of the decreased possibility, during multiple treatments, of peptide bond breakdown. Stepwise degradation of PTC-insulin in aqueous media in the presence of guanidine hydrochloride to keep the protein in solution was successful through several steps using 1N HCl at 36° (112) or 0.1N HCl at 75° (60). However, with dilute acid, besides the expected PTH's, increasing amounts of other PTH's were obtained in successive stages, indicating splitting of other peptide bonds.

The most successful procedure so far described for large PTC-peptides or proteins is due to Fraenkel-Conrat (110,114), who overcame the problem of insolubility of these compounds by carrying out all reactions on a paper strip so that a large surface of protein was accessible to volatile reagents.

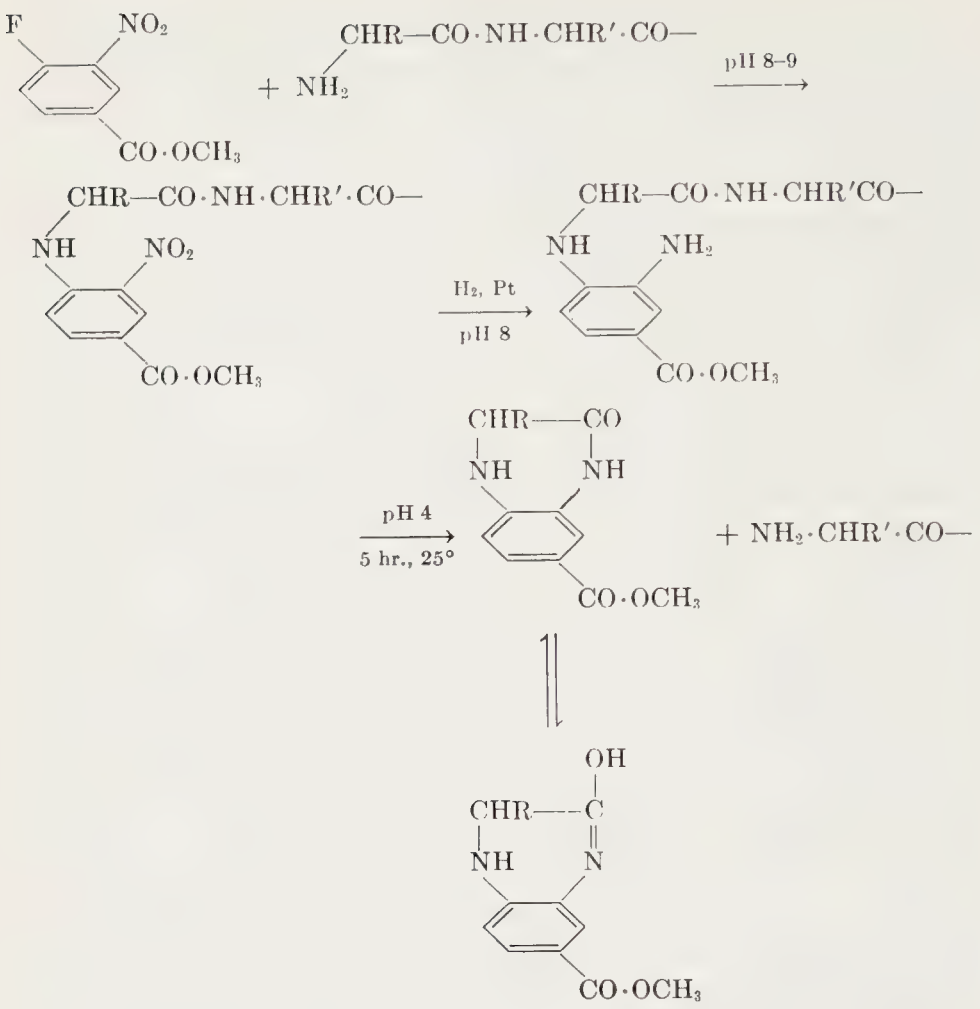
The cyclization is effected in an atmosphere of 6N HCl and glacial acetic acid at room temperature for 4–16 hr. All the PTH's are ex-

tractable from the paper, although half-cystine-PTH, when formed, will remain attached to the peptide chain. The yields of PTH's from successive steps have usually been high, averaging 80% based on the maximum optical density. However, lower yields on the basis of recovered products have been reported (121).

The most successful applications of this technique have been to the determination of the heptapeptide sequence H.Ser.Tyr.Ser.Met.Glu.-His.Phe- in sheep corticotropin (141), the decapaptide sequence H.Asp.Glu.Gly.Pro.Tyr.Lys.Met.Glu.His.Phe- in β-melanocyte-stimulating hormone (120,142), and a pentapeptide sequence H.Thr.Pro.-Val.Thr.Pro- in prolactin (63).

An alternative procedure using anhydrous trifluoroacetic acid (100), which has excellent solvent properties for proteins (172), has been applied successfully to the cyclization (5 min. at 0°) of PTC-pepsin through four steps H.Ileu.Glu.Asp.Glu- (90) and may be a suitable reagent for other PTC-proteins. In view of the rapid formation of the intermediate reactive thiazolinone (Fig. 17), it would seem preferable to extract this derivative after a short reaction period and complete the cyclization in 3*N* HCl which will not destroy PTH-serine or PTH-threonine. It is of interest that the extremely low recoveries found by Shepherd *et al.* (283) in their extensive degradative experiments on peptides from corticotropin were due to losses (50–70%) during acid cyclization in glacial acetic acid–HCl at 75–80° for 15 min. In view of the rapid formation of the thiazolinone and its reactive nature, these conditions would seem excessive. The experiments of these authors suggested that a critical phase of the decomposition occurs during the cleavage and/or cyclization, since the recovery under milder conditions was lower with PTC-peptides than the destruction of PTH-alanine under parallel conditions. This may account for the preference of some workers for a subtractive method (108,151,241) where the *N*-terminal amino acid is detected by its disappearance. In spite of low yields and random cleavages, Shepherd *et al.* (283) were able to detect the *N*-terminal residue, since it was usually present in 5- to 10-fold the amount of others. However, when an unstable, unextractable, or repeating amino acid is encountered, low yields do not inspire confidence and could lead to erroneous conclusions.

(*b*) *Other Methods.* Substituted *o*-nitrophenyl compounds are coupled to free amino groups by means of the corresponding fluoro



3-Substituted-7-carbomethoxy-2-hydroxydihydroquinoxaline

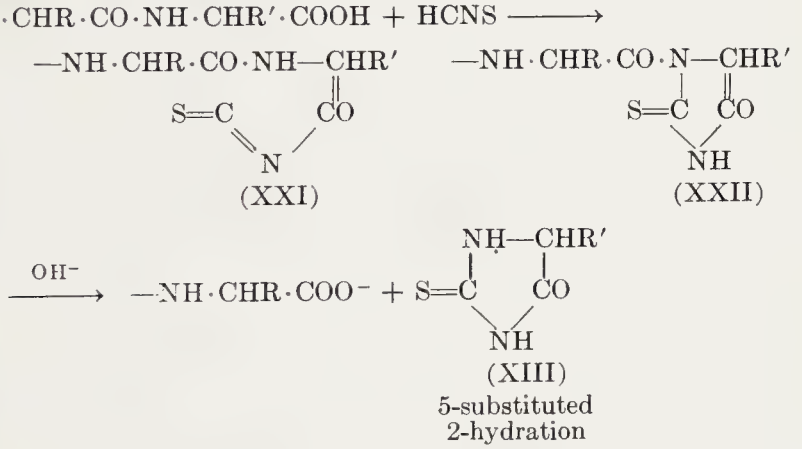
Fig. 19. Reactions involved in the Holley and Holley (155) method for stepwise degradation of peptides.

compound. Reduction of the nitro groups allows easy cyclization, under mild conditions, of the amino compound to a lactam. Holley and Holley (155) used 1-fluoro-2-nitro-4-carbomethoxybenzene in the development of this method with simple peptides (Fig. 19), but others (161,168) have attempted to use the more readily available 1-fluoro-2,4-dinitrobenzene. With DNP-peptides, it is necessary to work in the absence of oxygen to avoid secondary reactions, and the products are stabilized by dinitrophenylation. The DNP-aminodihydroxyquinoxalines can be identified chromatographically and the *N*-termi-

nal amino acid detected as its DNP derivative by hydrolysis of a portion of the new DNP-peptide. The advantage of this method is the mild cleavage conditions which are unlikely to lead to nonspecific peptide bond fission. However, when applied to natural products (161,168), only poor yields have so far been reported. A further modification involving selective reduction of the ortho nitro group has been described (280). The products are colored, more stable, and readily identified by paper chromatography. Details of the application of this method to peptides are not yet available.

2. Methods for C-Terminal Residues

(*a*) *Isothiocyanate Degradation.* The conversion of a *C*-terminal residue into an acyl isothiocyanate (XXI) facilitates spontaneous cyclization into an acyl thiohydantoin (XXII) (272).



The thiocyanic acid is usually obtained from acetic anhydride (which blocks the *N*-terminal group) and ammonium thiocyanate. Condensation occurs during gentle heating for short periods (19,300). Milder reaction conditions are possible using mixed anhydrides of thiocyanic acid (176,301), such as $(C_6H_5O)_2PO \cdot NCS$ (176).

Deacylation to a 5-substituted-2-thiohydantoin (XXIII) occurs readily in dilute alkali, e.g., 0.01*N* NaOH (176), or in acid (300), and may be followed spectrophotometrically (176,180).

The thiohydantoins may be identified by paper chromatography (68,94) or by regeneration to the amino acid (19) as for thiohydantoins obtained in the Edman method (Section V-B-1(a)).

This reaction is unsuited to *C*-terminal proline residues, which can-

not form a thiohydantoin; to aspartic and glutamic acids, which form cyclic anhydrides rather than thiohydantoins (whereas asparagine and glutamine will form thiohydantoins (300)); or to serine, threonine, cystine, arginine, and lysine (19,300), which are unstable during cyclization or regeneration of the amino acid. The applicability of the method to peptides or proteins is therefore severely limited as a direct procedure. As a subtractive method (107) the reaction may be more useful but should still fail for aspartic and glutamic acids and proline. However, with microbiological analyses (107) specific for L-residues, they might be recognized by the racemization of these three residues which results in a 50% loss when they are *C*-terminal.

(*b*) *Esterification, Reduction, and Acyl Shift of C-Terminal Residues.* An attractive scheme for stepwise degradation from the *C*-terminal residue was envisaged (15,65) which involved esterification and reduction of the carboxyl group with a metallohydride. An N–O acyl transformation on the reduction product (XXIV) would give the *C*-terminal residue linked by an easily hydrolyzable ester bond rather than by a peptide bond (XXV). This would have been split off as a β-amino alcohol (XXVII) by further reduction, leaving the residual peptide with a *C*-terminal β-hydroxyamide residue (XXVI) capable of another N–O acyl shift (14). The process could then be repeated.

$$\mathrm{R{\cdot}CO{\cdot}NH{\cdot}CHR'{\cdot}CONH{\cdot}CHR''{\cdot}COOCH_3} \xrightarrow{\text{reduction}}$$

$$\underset{\text{(XXIV)}}{\mathrm{R{\cdot}CO{\cdot}NH{\cdot}CHR'{\cdot}CO{\cdot}NH{\cdot}CHR''{\cdot}CH_2OH}} \xrightarrow{\text{acid or acid chloride}}$$

$$\underset{\text{(XXV)}}{\mathrm{R{\cdot}CO{\cdot}NH{\cdot}CHR'{\cdot}CO{\cdot}O{\cdot}CH_2{\cdot}CHR''{\cdot}NH_3^+}} \xrightarrow{\text{reduction}}$$

$$\underset{\text{(XXVI)}}{\mathrm{R{\cdot}CO{\cdot}NH{\cdot}CHR'{\cdot}CH_2OH}} + \underset{\text{(XXVII)}}{\mathrm{NH_2CHR''{\cdot}CH_2OH}}$$

A procedure such as this would have been particularly useful for peptides not containing serine or threonine residues (apart from *N*-terminal residues), such as would result from the fission of proteins by the acyl migration method (Section IV-B-1).

Difficulties were encountered, however, in both the reduction and rearrangement stages. The reduction of large peptides, insoluble in the reaction mixture, requires elevated temperatures for satisfactory reaction, and it was found (65) that reductive cleavage of peptide

bonds, particularly those involving the carboxyl groups of glycine and alanine, also occurred in the following way

$$—CO—NH— \longrightarrow —CH_2OH + NH_2—$$

This reaction has been acknowledged (58) as the cause of earlier anomalies in the determination of *C*-terminal amino acids in esterified insulin.

The difficulties encountered in the N—O acyl shift have already been discussed (Section IV-B-1). However, for small peptides soluble in organic solvents, the conditions worked out by Bailey (15) seem capable of successful application.

Methods for the isolation and estimation of the β-amino alcohols on a micro scale have been developed based on chromatographic separation and spectrophotometry of the DNP-amino alcohols (65,127,164, 167) or of the amino alcohols themselves, which can be estimated by periodate oxidation to formaldehyde (249).

VI. Concluding Remarks

In unravelling the amino acid sequence of peptides and proteins, some of the selective methods of degradation which have been surveyed are playing a dominant part. At the present time, only the most promising of them are being employed, since they are proving adequate in a rapidly advancing search for the relationship between structure and biological activity. However, for more complex proteins, other methods may be necessary. It is to be hoped that the challenge which these proteins present will stimulate continued exploration for new selective degradation methods. In these studies, knowledge of the amino acid sequence of an ever-increasing number of peptides will be particularly useful.

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OPTICAL ROTATORY DISPERSION AND THE STUDY OF ORGANIC STRUCTURES

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I. Introduction

The purpose of this review is to outline the use of optical rotatory dispersion—i.e., the variation of optical rotation with the wavelength

of light—in structural and stereochemical studies. Little will be said about theoretical approaches to the subject (for which see 117,150,153,160,178,137; also 79, ch. 12), since the empirical viewpoint is more valuable at present. No doubt in due time the empirical and theoretical approaches will converge, to their mutual advantage. At present, however, the best service which empirical workers can offer to their theoretical colleagues is to collect as wide a range of data as possible for subsequent interpretation.

The general principles relating rotatory dispersion and structure have been known for many years. In brief, for a compound which does not absorb light within the range of wavelengths studied, the magnitude of the rotation usually increases steadily with decreasing wavelength. For compounds which do absorb within the region studied, the curves are much more complicated, often showing one or more maxima and minima in the neighborhood of the absorption bands.

The use of dispersion measurements on an extensive scale for structural purposes is very recent. This sudden growth of the subject is due to the introduction of a commercially available photoelectric spectropolarimeter which permits the rapid determination of a large number of dispersion curves in a short time. Earlier workers were greatly handicapped by the tedious nature of the photographic measurements necessary; the determination of one curve required about three weeks' work. It is hoped that the development of an automatic recording polarimeter will make possible even more rapid advances in the future.

The present review is largely concerned with the newer work (1–31) which forms a connected study of the relations between dispersion and structure in a few well-defined fields. The more restricted studies of earlier workers are treated in less detail simply because the immense experimental difficulties made a broad view impossible; no disrespect for these laborious and careful studies (which are fully summarized by Lowry, 153, and by Levene and Rothen, 150) is intended.

In order to study the really striking features of dispersion curves, one must be able to measure the optical rotation of a substance right through an absorption band (cf. Kuhn, 136,138). This is experimentally possible only if the absorption band is in an accessible part of the spectrum and if the extinction coefficient is low. Up to the present, the carbonyl group (λ_{max} at 280–300 mμ; ϵ_{max} $\sim$50) has

proved the most convenient absorbing group which fulfills this specification. Compounds containing a ketonic or an aldehydic carbonyl group in an asymmetric environment (but not acids, esters, or lactones) give rotatory dispersion curves with very pronounced "maxima" and "minima" (now called "peaks" and "troughs").

Extensive application of the new photoelectric spectropolarimeter of Rudolph (186) to structural work has been made so far by only one school, that of Professor C. Djerassi, Stanford University, California (1–31). This work has been largely concerned with cyclic ketones for the reasons outlined above. The author is greatly indebted to Professor Djerassi for personal discussions and correspondence, and for the communication of many unpublished data. Djerassi has published two reviews of his work (11,78) and recently a full-length book (79).

It is certain that for absorbing compounds the position and intensity of the "maxima" and "minima" will be of much greater value for structural purposes than the "monochromatic" measurements—usually for the D line—which have previously been used (for reviews of "monochromatic" rotation measurements in structural studies, see 130,132,163). For nonabsorbing compounds, the position is less clear; it may well be that the shapes of curves, or the ratio of rotations at two wavelengths ($[\phi]_1/[\phi]_2$),* or even a single rotation value at some wavelength other than the D line, may be of greater value than the classical $[\phi]_D$; we should, however, keep an open mind until more extensive studies on nonabsorbing compounds have been made.

Limitations of space preclude a discussion in this review of the use of rotatory dispersion measurements in the study of high polymers including proteins (cf. 93,94,115,125,168,169; especially 201; also 79, ch. 17).

This review was originally completed in July, 1958; the last section (VI) deals with the one major development in the field since this date. Some few examples of individual compounds studied between July, 1958, and October, 1959, have been included—but no attempt has been made to include all the literature over this period.

II. Apparatus

A. CLASSICAL METHODS

Nearly all the instruments used for the measurement of rotatory dispersions before 1950 were elaborate, and their use involved much

* $[\phi]$ is now preferred to $[M]$ as a symbol for molecular rotation.

tedious photography and photometry of spectra. Furthermore, few if any were commercially available, and it is hardly surprising that the work was the province of specialists who built their own equipment. Even if these earlier spectropolarimeters had been on the market, the time required to measure a single dispersion curve was such that they could never have become a tool for routine structural or analytical studies.

Detailed accounts of the various classical instruments are given by Heller (110) and by Lowry (153); leading references, for equipment, together with the users, (135 Kuhn and Levene; 156 Lowry; 103, Hargreaves).

B. PRESENT-DAY SPECTROPOLARIMETERS

Rudolph (186) introduced in 1955 a photoelectric spectropolarimeter which is in the simplest terms, an orthodox high precision polarimeter (quartz optics) with a monochromator to provide light of different wavelengths, in place of a sodium lamp, and a phototube and photomultiplier, in place of the human eye, to measure the angle of rotation. This equipment has permitted one single school (that of Djerassi) to amass in six years dispersion curves for over 2000 compounds, probably more than have been measured in the whole previous history of polarimetry. About 50 other photoelectric spectropolarimeters are now in use.

Instruments of improved optical design with automatic recorders are in course of development (see Section II-C), but, however great the advantages of these instruments may be in future years, they cannot take from the late Prof. E. Brandt (Columbia) and the Rudolph Company the credit of having designed and developed the first spectropolarimeter suitable for routine use by organic chemists (cf. 60).

The following details of the Rudolph equipment and its use are taken largely from Djerassi (1). The Rudolph photoelectric spectropolarimeter model 200S-80 consists of four components: Rudolph circular scale high precision polarimeter (model 80), a Beckman quartz monochromator, light sources, and a Photovolt Multiplier Photometer (model 520-M) with an ultraviolet photomultiplier tube (RCA IP28). The light sources used by Djerassi have been (*a*) (700–300 mμ) a glass-jacketed Sylvania K-100 concentrated arc Zirconium lamp, and (*b*) (below 300 mμ) a 150 watt xenon arc lamp

(Hanovia 10-C-1), which was operated on direct current with a special commercially available power supply unit.

The speed of operation may be greatly increased by the use of an oscillating polarizer (Rudolph no. 200A) (187). An experienced operator can now plot a curve in $^1/_2$–1 hr.

The polarimeter tube used is of the center-filling type with cemented-on fused quartz cover glasses (1 dm. length; capacity <2 ml.). Solutions are generally of 1 or 0.1% concentration; perhaps less for strongly absorbing compounds.

Readings are taken in duplicate, usually by the method of symmetrical angles, and blank readings with pure solvent are performed daily. The solvents used for work with alicylic ketones have been chiefly methanol and dioxane, occasionally chloroform, ethanol, and octane.

For compounds not absorbing above 400 mμ, the following wavelengths are convenient for measurement—700, 650, 620, 589 (sodium D line), 550, 520–400 (in 20 mμ intervals) and 400–250 mμ (in 5 or 10 mμ intervals); in the region of "peaks" and "troughs," readings should be carried out at 2.5 mμ intervals or closer.

Duplicate readings should agree generally within ±0.002°, except at the lower wavelengths where larger deviations are encountered and more than two readings are taken. Agreement between results for two different solutions made up from the same sample should be within 3% (except for very dilute solutions where it may be up to 10%).

A Rudolph polarimeter has recently been installed in the author's laboratory in combination with a Unicam SP500 monochromator in place of the Beckman model and with a Siemens 375 watt xenon arc as light source.

Another type of spectropolarimeter (apparently suitable only for the range 400–700 mμ and therefore of limited value in structural work) is the Keston Unit (Standard Polarimeter Co., 225 E. 54 Street, New York, N. Y.), which may be attached to the Beckman model DU spectrophotometer.

C. INSTRUMENTS UNDER CONSTRUCTION OR DEVELOPMENT

Chief interest centers on the means of measuring the rotation; the following very condensed account is necessarily incomplete since many of the instruments mentioned are still in the prototype or development stage. The devices fall into three classes.

1. *Mechanical movement of one of the optical components.* Two automatic recording polarimeters use mechanical rotation of the

polarizer or analyzer. In the Rudolph Recording Spectropolarimeter the polarizer is rotated to compensate for the sample rotation, and the angle through which the polarizer is turned is plotted mechanically against wavelength. In the Unicam instrument (which is based on the Glaxo design, see below) one of a pair of Wollaston prisms is rotated for the same purpose.

2. *Sample rotation balanced by Faraday effect.* In several types of polarimeter the sample rotation is compensated by an equal and opposite rotation produced electromagnetically in a Faraday cell. The current necessary to produce the balancing rotation is related to the angle of rotation (Gillham, 101; Gates, 98; Grosjean *et al.*, 102a; also Cary and Zeiss instruments in preparation and a recording instrument under development at the National Physical Laboratory (J. N. Gates and R. J. King)).

3. *Symmetrical angle principle: difference in intensity of two beams polarized in different planes.* If two beams polarized in different planes (angles, $\pm\vartheta$ to a zero plane) are passed through an optically inactive material and then through an analyzer set in the zero plane, the intensities of the two beams are equal. If now an optically active sample is put in place of the inactive material, the planes of vibration of the beams emerging from it will be $(+\vartheta + \alpha)$ and $(+\vartheta - \alpha)$; when these have passed through the analyzer in the zero plane, their intensities will *not* be equal and the ratio of their intensities will be related to α, the rotation of the sample. The measuring problem resolves itself into the measurement of a ratio of two currents (when the two beams fall on a photocell). Several newer polarimeters depend on this principle, the two beams being separated "in time" (by putting the $+\vartheta$ and $-\vartheta$ beams through the sample alternately) or "in space" (by putting the two beams through two identical samples). Examples of the first type (time) are the instruments of Bürer, Kohler, and Günthard (64a; Zürich) and of Fordyce, Parker, and Green (95; Glaxo); of the second type (space) those of Perkin-Elmer (187a) and of Woldbye (197a).

III. Results

A. CLASSIFICATION OF CURVES

1. Simple and Complex Dispersion Curves

For many compounds which do not absorb light within the range

of wavelengths measured, the relation between $[\alpha]$ and λ is given by the simple equation

$$[\alpha] = A_0/(\lambda^2 - \lambda_0^2)$$

where A_0 and λ_0 are constants for a given compound. This is called a one-term Drude equation, and the dispersion is called *simple* (Lowry, 153). In nonmathematical terms, the plot of $[\alpha]$ against λ is a smooth curve, $[\alpha]$ increasing as λ decreases (see Fig. 1).

In other cases

$$[\alpha] = A_0/(\lambda^2 - \lambda_0^2) + A_1/(\lambda^2 - \lambda^2) + \ldots$$

This is called a two-term, three-term, or many-term Drude equation, and the plot of $[\alpha]$ against λ is a complicated curve which may have maxima, minima, and inflections. This sort of dispersion curve is called *complex;* the "maxima" and "minima" in the dispersion curve are often close to the wavelengths of absorption maxima.

For alternative treatments see Yang and Doty (201) and Heller (110a).

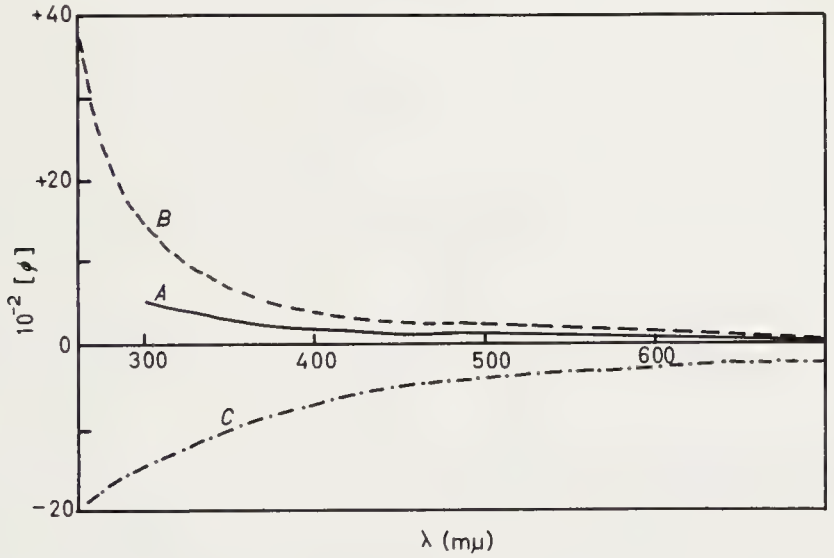


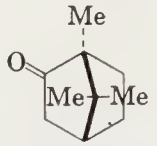
Fig. 1. Plain curves. *A*, 5α-Cholestan-3β-ol (II) in dioxane; *B*, methyl 3β-hydroxy-5α-etianate in methanol; *C*, 5α-spirostan (XX) in dioxane.

2. Normal and Anomalous Dispersion Curves

Two other terms which have been used are "normal" and "anomalous" curves; a *normal* curve is one without a maximum (or mini-

mum), a zero value, or a point of inflection; an *anomalous* curve is one which shows one or more of these features (Lowry, 153). It may be noted that "normal" and "simple" are not synonymous, but "normal" includes "simple." "Anomalous" and "complex" are not synonymous, but "complex" includes "anomalous."

A classical example (camphor, I), illustrating the relationship of the absorption maximum to the dispersion curve, is shown in Figure 2 (Kuhn and Gore, 142).



(I)
(+)-Camphor

*3. Empirical Classification of Curves and Nomenclature Used in Recent Work**

Difficulties have been experienced in fitting mathematical equations (3) to many of the dispersion curves found in the work at Wayne State University. Attention has therefore been concentrated for the present on empirical studies of the shapes of the curves, since these are of considerable value in structural and stereochemical studies. It is for this reason that we have deliberately chosen an entirely new set of terms which can be used to describe curves without their reproduction. If in the future it is possible to analyze the absorption regions of the curves mathematically, other and more precise terms may be appropriate.

Three principal types of rotatory dispersion curves (abbreviated R.D. curves) must be considered in the present state of our knowledge—each in two antipodal (enantiomeric or mirror image) forms (Figs. 1, 4, and 9).

It is desirable to indicate in each paper (or series of papers) the intervals between wavelengths at which readings are taken (2) and the magnitude of the probable error (8, fn. 34). Solvent, concentration, and temperature of measurement can often conveniently be stated at the beginning of the experimental section.

* This section is copied almost verbatim from Djerassi and Klyne (84) by kind permission of the Publication Committee of the Chemical Society. It has been somewhat modified to correspond with proposals now under consideration by the Molecular Spectroscopy Commission of IUPAC.

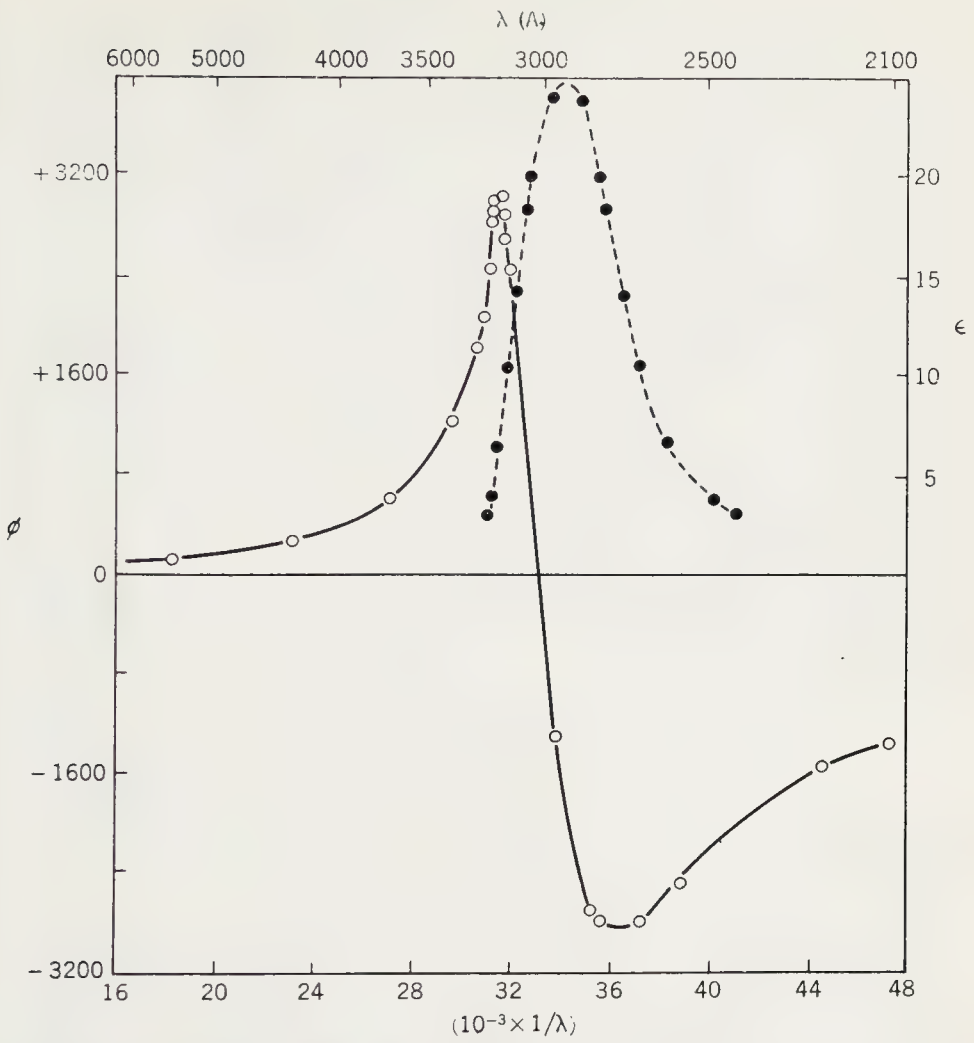


Fig. 2 Absorption and molecular rotation of camphor (I) in hexane. O——O, molecular rotation [ϕ]; ●----●, molecular extinction coefficient, ϵ. (From Kuhn and Gore, 142.)

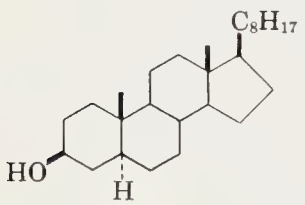
(*a*) *Plain Curves* (Fig. 1). The first type of curve shows no maximum or minimum* and is typical of compounds which have no optically active absorption bands within the wavelength range under consideration. For these we suggest the name *plain* curves, regardless of whether or not they can be expressed by a one-term Drude equation. At the present time, their chief utility lies in the fact that

* Or only a broad maximum or minimum which is not associated with an absorption band. A few curves cross the axis [ϕ] = 0; i.e., the rotation changes sign.

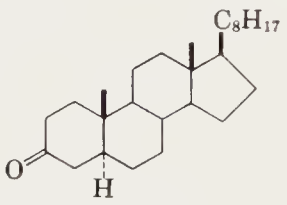
rotations in the ultraviolet region are (for colorless compounds) invariably greater than for the sodium D line; comparisons of compounds with small $[\alpha]_D$ are therefore better carried out at a lower wavelength, which can be selected from the dispersion curve. These curves may be called *positive* or *negative* according as they rise or fall toward *shorter* wavelengths. They may be adequately described in the text without a figure in the following terms:

Specific rotation, $[\alpha]$, or molecular rotation, $[\phi]$, at the following wavelengths: (*1*) the longest wavelength measured—usually 700 mμ; (*2*) 589 mμ, the Na D line, used in nearly all previous correlations between rotation and structure; and (*3*) the shortest wavelength measured. If desired, other points may be included; 100 mμ intervals would be convenient. Solvent and concentration will also be stated if this has not been done in a general description.

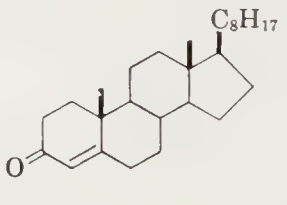
Example 1. 5α-Cholestan-3β-ol (II) (3): R.D. in dioxane (c = 1.0%), 23–25°C.; positive plain curve: $[\alpha]_{700}$, +17°; $[\alpha]_{589}$, +24°; $[\alpha]_{290}$, +130°.*



(II)
5α-Cholestan-3β-ol



(III)
5α-Cholestan-3-one



(IV)
Cholest-4-en-3-one

(*b*) *Single Cotton Effect Curves.* Of particular interest in structural and stereochemical studies are anomalous dispersion curves which are usually of two types (Figs. 4 and 9). Figure 4 shows examples of curves exhibiting a single Cotton effect (73), each with one geometrical "maximum" and one geometrical "minimum." The region of the wave including the "maximum" and "minimum" coincides more or less exactly with an absorption band. The curves

* Regarding one matter of detail, there is a clear difference of opinion between the British and the United States chemists with whom this matter has been discussed. Most United States workers like to have the wavelengths shown as subscripts, while most British workers prefer an alternative method in which the wavelengths are given in normal-sized italic figures in parentheses, and the temperature as superscripts, i.e., $[\alpha]^{23-25}$ (*700* mμ) +17°, (*589*) +24, (*290*) +130° (see *Helv. Chim. Acta*, *41*, 250 (1958)).

are called *positive* (or *negative*) when the "wave" is entered from the positive (or negative) side going toward *shorter* wavelengths. The terms *peak* and *trough* are suggested instead of "maximum" and "minimum," since the latter are often misleading, and these new terms should not be confused with absorption maxima and minima in visible or ultraviolet spectra. German equivalents (*Gipfel, Tal*) and French equivalents (*sommet, creux*) have been proposed by Professor V. Prelog and Dr. E. Heilbronner and by Dr. G. Ourisson, respectively. The vertical distance between peak and trough (*a* in Fig. 4) may be called the amplitude of the wave.

A description of such a curve should include the rotations at 700 mμ, at 589 mμ, at the shortest wavelength measured, and at each marked change of direction, i.e., peaks, troughs, and such minor features as shoulders or inflections. All these rotations should be given in order of decreasing wavelength.

Example 2. 5α-Cholestan-3-one (III) (5): R.D. in methanol (c = 0.1%), 29–31°C.; $[\alpha]_{700}$, +37°; $[\alpha]_{589}$, +55°; $[\alpha]_{307}$, +959°; $[\alpha]_{207}$, −740°; $[\alpha]_{245}$, −362° (curve *A*, Fig. 4).

It must be emphasized that these data do not indicate the wavelength intervals between readings (1).

(*c*) *Multiple Cotton Effect Curves.* The third type of curve is more complicated in the region of the absorption band, with two or more peaks and a corresponding number of troughs; it will be called a *multiple Cotton effect curve* (Fig. 9)—cf. the classic example of octyl nitrite studied by Kuhn and his colleagues (143). It may sometimes be necessary to distinguish broad peaks and troughs, also shoulders and inflections, as in the following example.

Example 3. Cholest-4-en-3-one (IV) (8): R.D. in dioxane (c = 1.0%, 29–31°C.; $[\alpha]_{700}$, +53°; $[\alpha]_{589}$, +73°; $[\alpha]_{425-410}$, +145° (broad peak); $[\alpha]_{366}$, −93°; $[\alpha]_{359}$, −51°; $[\alpha]_{352}$, −164°; $[\alpha]_{340-337}$, +265° (shoulder); $[\alpha]_{325}$, +1000° (inflection); $[\alpha]_{311}$, +1480° (inflection); $[\alpha]_{275}$, + 2100°.

(*d*) *Extrema.* A term is needed which will cover both *peaks* and *troughs.* The term *extremum* was suggested for this purpose by the late Dr. W. Moffitt. It is often necessary to designate the peak (or trough) on the higher wavelength side of a Cotton effect curve—e.g., the peak (or trough) at about 310 mμ for a saturated ketone; for this, the term *first extremum* is suggested.

4. Use of Molecular Rotatory Dispersion Curves

Nearly all the data presented so far in the papers of Djerassi *et al.* have been expressed as *specific rotations.* Professor Djerassi (personal communication) has stated that this has been done in order to emphasize that the values of $[\alpha]$ in the region of absorption bands are very large compared with the small $[\alpha]_D$ values to which organic chemists are accustomed.

In the present author's view the time has come when all rotatory dispersion curves should be expressed in terms of *molecular rotations,* $[\phi]$, defined as

$$[\phi] = [\alpha] \times \text{mol. wt.}/100$$

in order that quantitative comparisons between related compounds and the addition and subtraction of rotation curves can readily by carried out (85,131). For this reason all data and curves in this review (with very few exceptions) are expressed as $[\phi]$, *not* $[\alpha]$. The symbol $[\phi]$, proposed by late Dr. W. Moffitt (Harvard), is now preferred to $[M]$ for molecular rotations.

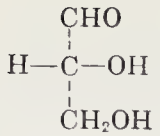
5. Alternative Methods of Plotting Data

In the classical studies on rotatory dispersion, many methods of plotting were used to test the fit of experimental data with an equation. The method which seems to offer most promise for structural studies, in the region of transparency, is that in which the *reciprocal* of the rotation, $1/[\phi]$, is plotted against the *square* of the wavelength, λ^2. Compounds which, for the whole or part of the spectrum studied, have one-term Drude equations give straight line plots of $1/[\phi]$ against λ^2 (for examples and fuller treatment see Lowry, 153).

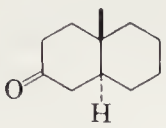
Another method recently introduced by Yang and Doty (201) is to plot $\lambda^2[\phi]$ against $[\phi]$; this also gives a straight line plot for the one-term Drude equation. See also Heller (110a).

6. Formulas and Conventions

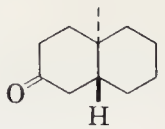
All formulas show known absolute configurations unless indicated to the contrary. Acyclic formulas follow the usual Fischer convention (now known (55) to be correct in absolute terms), e.g., V for D-glyceraldehyde.



(V)



(VI)



(VII = EVI)

Cyclic formulas follow steroid conventions (also known to be correct in absolute terms), e.g., VI for the methyldecalone corresponding to 5α-cholestan-3-one (III). Bonds above and below the plane of the paper are shown by thick and dashed lines, respectively.

(*a*) *Enantiomers.* In order to facilitate comparisons, use will frequently be made of such expressions as "*A* showed that the dispersion curve of (+)-X was . . ." when, in fact, *A* studied experimentally the (−)-enantiomer, and the signs of all rotations have been changed.

The letter E is used to indicate "enantiomer of"; e.g., VII may be referred to as EVI.

B. "CLASSICAL" WORK

The rotatory dispersion curves of many organic compounds of widely differing types were measured with the older instruments available before the introduction of photoelectric spectropolarmeters. Much of this classical work is summarized in Lowry's book (153) and the review by Levene and Rothen (150). (See also two symposia of the Faraday Society, 192 and 193.) On account of instrumental difficulties, the number of compounds which could be studied per man-year was small; and, since the earlier workers were largely physical chemists, they concentrated on a few compounds in their studies on the fundamentals of rotatory dispersion. Valuable as this earlier work was in laying and testing the theoretical foundations of the subject, it did not, in the present author's opinion, provide much information which might be of value in structural and stereochemical studies.

In particular, the efforts to fit one-term or two-term Drude equations to the experimental data were by no means always rewarding. In many cases one equation was found in early work to cover the visible spectrum, and subsequent ultraviolet measurements showed that a more complex equation was necessary. In some cases the constants (λ_0, λ_1, λ_2) found for the Drude equations bore a close relation to the absorption maxima of functional groups in the compound; in others they did not.

For these reasons no details of the dispersion curves obtained in the

TABLE I
Classical Work

Compound or class	References		
	Lowry (153), page	Levene and Rothen (150), page	Original literature
Hydrocarbons, saturated		1805–7	121a,149
Hydrocarbons, unsaturated	127		
Hydrocarbons, aromatic, nonplanar			105
Halogen derivatives[a] (see also p. 261)		1824–28	148
Alcohols, primary ($RR'CH \cdot CH_2OH$)	265–66		121a
Alcohols, secondary[b]			
General	265–67	1805, 1807	
Heptan-2-ol			121e
Octan-2-ol	130	1810–11	103,138
Octan-4-ol		1805	
Cyclohexylmethylcarbinol		1810–11	138
Menthol and other terpene alcohols	128–129		
Alkylaryl carbinols and related alcohols	265–66	1810–11	42,92a,100, 104,122,138
Heteroaromatic carbinols			35,36,43
Miscellaneous			32,44,121d
Alcohols, unsaturated		1813	51,52,[c]120
Ethers (incl. benzyl)	263,267	1806	44
Aldehydes[a] (see also pp. 261–262)			
2-Methylbutanal and 3-methylpentanal		1820–22	112,147
Citronellal			102b
Ketones[a]			
Aliphatic			121c,175
Acetoin			154
3-Methylcyclohexanone			97
Pulegone, carvone	128		
Menthone, carvomenthone			159
Camphor and derivatives	128–29,141, 146,305–12,405–07		142,189
Methylenecamphor	128		102a
Hydroxymethylenecamphor	119		
Hydroxymethylenecamphor, metal derivs.	156		
Camphorquinone	134–35,145, 156,309–10		

TABLE I (*continued*)

Compound or class	References		
	Lowry (153), page	Levene and Rothen (150), page	Original literature
Santonides			167,199
Dibenzylidenemethylcyclohexanones			173,174
Acids and their esters (incl. 2-methylbutanoic and 3-methylpentanoic acids)	265–68	1814–17, 1819–20	146[d] 121[a]
Phenyl-substituted[a,c]		1817–19	37,37a, 149,172, 176
Quaternary $BuMePhC \cdot CO_2H$			37
Dicarboxylic			190a
Halogen-substituted acids (especially α-halogen propionic acids, esters, and dimethylamides)	269, 439–40	1835–40	139–141
Hydroxy acids and derivatives			118
Lactic acid	298–301		138[f]
Malic acid	295–98		
Tartaric acid (incl. borate complexes)	108,136–40, 144 286–93		107,198[g]
Metallic derivatives[h]	154–56		
Mandelic acid, derivs.[a,i]		1819–20	
Mercapto acids	269	1831–34	
Amines ($BuMePhC \cdot NH_2$)			37
Amine oxide			96
Amino acids (see pp. 255–257)			60,108,119, 138[f]
Copper derivatives (see pp. 306–308)			196
Schiff bases ($ArCH{:}N \cdot CHRR'$)			54,171
Peptides			60
Xanthates and other derivatives of thiocarbonic acid[a] (see also p. 262)	318–25,398, 401,403, 445,447		158,194
Azides		1822–23	149
Azido acids[a,j] (see also p. 261)			
α-Azidopropionic acid and derivatives[k]	269,397,434, 439	1829–31	140
Sulfonic–carboxylic acids	269	1831–34	
Nitrites[a] (see also pp. 259, 262)			

(*continued*)

TABLE I (*continued*)

Compound or class	References		
	Lowry (153), page	Levene and Rothen (150), page	Original literature
Octan-2-ol and nonan-3-ol	145,441–44	1810–11	143,138[l]
Cyclohexyl and phenylmethyl carbinols		1810–11	138
Nitrosites	157–8		164,165[m]
Dinitrocamphene			166
Nitrocamphor, metal derivatives	156		
Carbohydrates[a] (see also p. 257)			
Aldehydo sugars	133–34,283–85		39,112
Galactonic acid and lactone	281–82		
Sucrose	131,279–80		
Azophenylglucosides			57
Nitriles and thioketals			39
Adenosine and derivatives			145
Heterocyclic compounds			
O-Derivatives of malic and tartaric acids	290,297		
Ethylene oxides			44,106
Nicotine and derivatives	329–33		
Tetrapyrrolidines (bile pigments)			102
Cytochrome *c* (asymmetric Fe)			91
Spiran (4-methylcyclohexylidene acetic acid)	334		
Nonplanar aromatic hydrocarbon			105
Cobalt ethylenediamine complexes			160a
Sulfoxides and sulfilimines			111c

[a] See also Table II.

[b] Many references include esters.

[c] These recent Russian papers describe a photographic spectropolarimeter which appears to be based on the design of Cotton and Descamps (74).

[d] Effect of distance of CO_2H from asymmetric center discussed.

[e] Absorption band at 260 mμ *not* active (contrast ethyl mandelate).

[f] Comparison of alanine and lactic acid.

[g] For discussion regarding complexity of dispersion see Lowry pp. 291–93.

[h] Includes Cotton's original work on potassium chromium tartrate (73).

[i] Absorption band at 260 mμ active.

[j] Absorption band at 288 mμ *not* active.

[k] Absorption band at 288 mμ active.

[l] Detailed analysis in this paper.

[m] Note temperature effects.

classical work for nonabsorbing compounds will be given here. Table I gives a list of compounds or classes studied, with references to Lowry and/or Levene and Rothen, where appropriate, and to the original literature for a few key papers and for later work. No doubt in a few years' time when a much wider range of nonabsorbing compounds has been studied by the rapid methods now available these pioneer studies will find their true place.

Recent published work on plain curves includes studies on aralkyl compounds of the types VIIa (Mateos and Cram, 159b) and VIIb (Lyle, 159a; some show Cotton effects), monohydroxysteroids and their esters (Jones and Klyne, 116). Work in progress in the author's laboratory includes surveys of lignans (with H. Erdtman, Stockholm) and of ureidoamino acids and sulfoxides (with A. Kjaer, Copenhagen).

$$\underset{\text{(VIIa)}}{\mathrm{Ar\cdot CHR\cdot CHOH\cdot R'}} \qquad \underset{\text{(VIIb)}}{\mathrm{Ar\cdot CH(NH_2)\cdot R}}$$

A few scattered references to work with spectropolarimeters other than that of Djerassi's school are included here for convenience.

The only series of compounds giving "plain" curves where sufficient examples have been studied to permit the use of these curves for stereochemical studies is the series of α-amino acids.

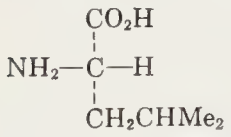
1. Amino Acids

Patterson and Brode (179) studied a selection of the natural amino acids at 660–440 mμ and deduced generalizations which might be used for allotment of configurations. Greenstein and his colleagues (113,114,177) subsequently extended this work to a series of 42 L-amino acids which were studied at 589–365 mμ.

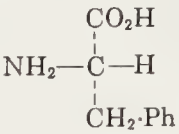
The L-amino acids may be classified into three groups represented by the curves in Figure 3 (based on ref. 179), in which $1/[\phi]$ is plotted against λ^2. Curve *A* (L-leucine hydrochloride) is a typical normal positive dispersion curve cutting the x axis above $(200\ \mathrm{m}\mu)^2$. Most of the nonaromatic L-amino acids and their salts follow this pattern. Curve *B* (L-leucine (VIII) in water) is a normal negative dispersion curve cutting the x axis below $(160\ \mathrm{m}\mu)^2$.* About ten of the L-compounds examined show this pattern, chiefly aromatic and hydroxy-

* Greenstein suggests 200 mμ as the upper limit for Group *B;* there is, however, only one Group *B* amino acid with λ_0 above about 160 mμ, viz., L-cystine in hydrochloric acid (198 mμ). This is an unusual amino acid in many respects.

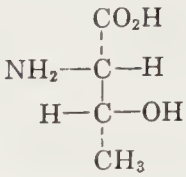
amino acids. Curve *C* is an anomalous dispersion curve (L-phenylalanine (IX) in sodium hydroxide); the rotation changes from negative to positive with decreasing wavelength. A few aromatic amino acids show this behavior.



(VIII)
L-Leucine



(IX)
L-Phenylalanine



(X)
L-Threonine

D-Amino acids would show dispersion curves which are the mirror image of those in Figure 3, and the form of curve for an unknown amino acid can therefore be used to allot it to the D- or L-series.

Greenstein wisely states (177) that "it is difficult to assign a particular physical significance to values of λ_0 when the experimental data are obtained at wave lengths so relatively far from the region of absorption, but it is of interest to observe the apparent regularities associated with this figure."

The Bethesda group (177) then considered the contributions of the α-centers in diasymmetric amino acids (e.g., X). These contributions were calculated as follows:

$$\text{Contribution of } \alpha\text{-center in L-form} = [\phi](\alpha\text{L})$$
$$= {}^1/_2\{[\phi](\text{L}) + [\phi](\text{L-allo})\}$$

$[\phi](\text{L})$ and $[\phi](\text{L-allo})$ are the molecular rotations of the L- and L-allo diastereoisomers at a given wavelength. The modified Patterson-Brode rules were found to apply (in most cases) to the contributions of α-centers when treated in this way.

The Cotton effect curves given by the copper derivatives of the amino acids are discussed on pages 306–308.

2. Carbohydrates

Extensive studies were carried out on a number of carbohydrates during the period 1932–37 by Hirst, Wood, and other members of the Birmingham school (111b). In view of the wide application of "monochromatic" rotation studies in the carbohydrate field, notably by C. S. Hudson, it is to be expected that the extension with more

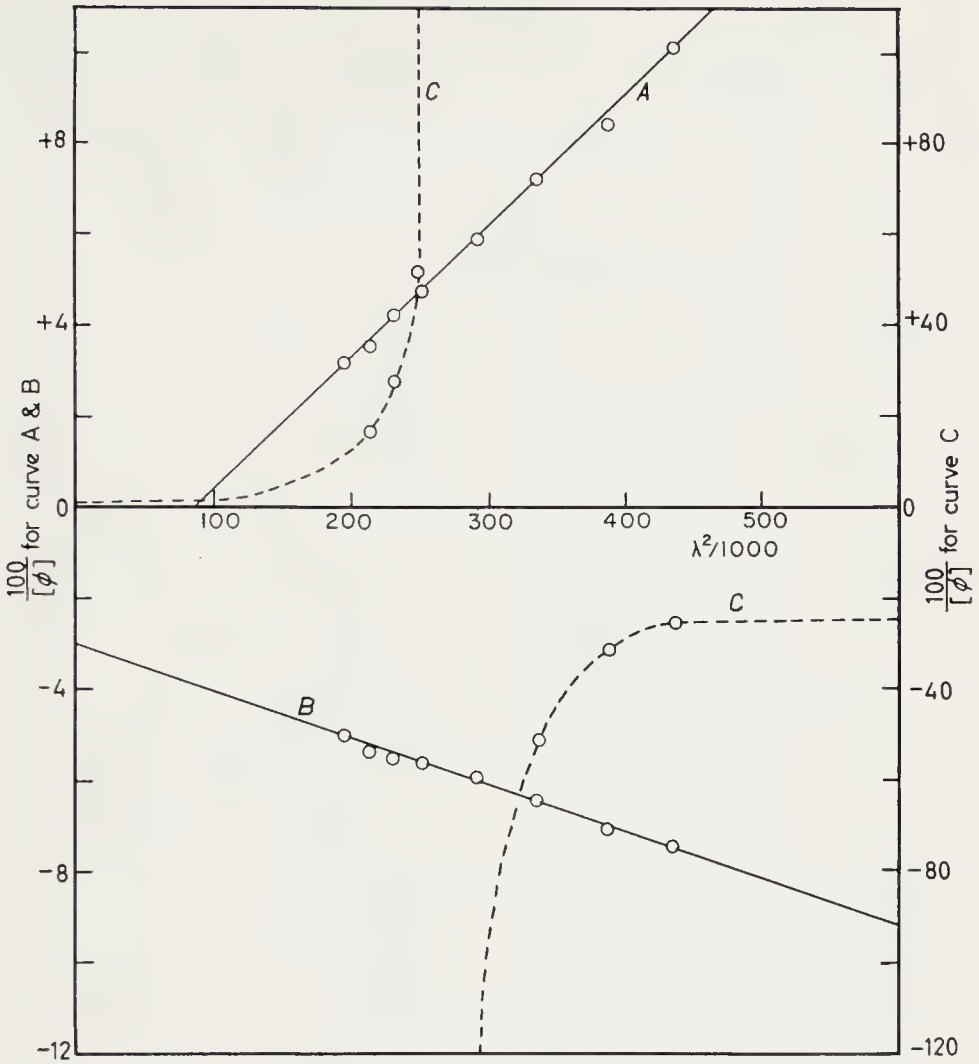


Fig. 3. Dispersion of L-α-amino acids, plotted as "Drude curves" (reciprocal of rotation against square of wavelength). *A*, L-Leucine (VII) in HCl; *B*, L-leucine in water; *C*, L-phenylalanine (IX) in NaOH (ordinate scale at *right* of figure refers to curve *C* only). (From Patterson and Brode, 179, Figs. 1 and 2.)

modern equipment of this pioneer dispersion work will prove fruitful (see also refs. in Table I).

3. Absorbing Compounds

The few compounds for which the older workers were able to follow the dispersion curve into or through an absorption band are listed in

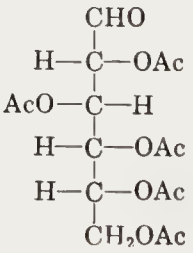
TABLE II

Absorbing Compounds Studied in Classical Work

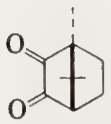
([ϕ] and λ values are for peaks and troughs except for those shown in *italics*, which are for the lowest wavelength measured.)

Compound	Solvent[a]	Peaks and troughs [ϕ]	Peaks and troughs λ, mμ	λ abs. max., mμ	Ref.
Halogen Derivatives					
α-Chloropropionic acid, methyl ester	HC	+ 1,800	250	~220	141
		− 3,500	215		
α-Bromopropionic acid Ethyl ester	HC	+ 3,900	260	~240	140
		− 5,500	220		
Dimethylamide	HC	+ *1,400*	295	~240	140
α-Iodopropionic acid Ethyl ester	HC	+ *4,500*	260	280	139
Dimethylamide	HC	− *200*	260		
2-Iodooctane	HC	+ *1,000*	300	265	139
Aldehydes and Aldehydo Sugars					
2-Methylbutan-1-al	HC	+ 470	320	295	147
		− 450	277		
3-Methylpentan-1-al	HC	− 580	320	295	147
		+ 1,100	273		
Campholic aldehyde (XI)	C	+ 1,460	322	295	155
		− 770	275		
Aldehydo-D-glucose penta-acetate (XII)		− 1,810	312	292	112
		+ 2,940	260		
Aldehydo-D-galactose penta-acetate	C	− 4,250	310	290	112
		+ 5,540	265		
Aldehydo-L-arabinose penta-acetate	C	− 3,630	312	290	112
		+ 3,630	268		

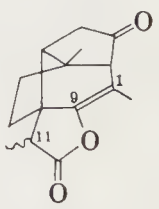
(XI)
Campholic aldehyde



(XII)
Aldehydo-D-glucose pentaacetate



(XIII)
Camphorquinone



(XIV)
Santonide

TABLE II (*continued*)

Compound	Solvent[a]	Peaks and troughs [ϕ]	Peaks and troughs λ, mμ	λ abs. max., mμ	Ref.
		Ketones			
3-Methylcyclohexanone	HC	+ 1,290	319	289	97
		− 1,580	263		
Menthone	HC	− 242	302	292	159
		− 72	271		
Carvomenthone	HC	− 868	320	288	159
		+ 1,170	277		
Camphor (I)	HC	+ 3,950	320	292	157
		− 3,340	272		
Camphorquinone (XIII)	HC	− 750	494	467	157
		+ 500	444		
Santonide (XIV)	A	+62,700	321	300	167; cf.
		−76,400	275		199
Parasantonide[b]	A	+78,800	321	300	167; cf.
		−86,000	275		199
		Phenyl-Substituted Alcohols and Acids			
Methylphenylcarbinol	HC	− *650*	275	~260	138
Methylphenylcarbinol acetate	HC	− *5,000*	238	~260	138
Mandelic acid					
Ethyl ester	A	−*13,000*	260	~260	138
Nitrile	HC–E	+ *1,800*	232	~260	138
Me ether, ester	HC	−12,500	237	~260	138
Me ether, dimethylamide	HC	+21,000	250	~260	138
Atrolactic acid					
Me ether, Me ester	HC	+10,400	242	~250	138
Me ether, dimethylamide	HC	+17,000	255	~250	138
		Azido Derivatives			
α-Azidopropionic acid	A(?)	− 95	310	283	140
Methyl ester		+ 320	267		
Dimethylamide	E	− 3,050	305[c]	290	140
		− 2,500	278		
		Nitrites, Nitrosites, and Nitro Compounds			
(+)-Methyl-*n*-hexylcarbinol nitrite	HC	Four peaks and four troughs			
		− 800	375	400–330	138

(*continued*)

TABLE II (*continued*)

Compound	Solvent[a]	Peaks and troughs [ϕ]	Peaks and troughs λ, mμ	λ abs. max., mμ	Ref.
(+)-Methylcyclohexylcarbinol nitrite	HC	Four peaks and four troughs			
		− 1,100	375	400–340	138
(−)-Methylphenylcarbinol nitrite	HC	Three peaks and three troughs[d]			
		− 820	365	400–350	138
		+ 550	375		
Bornylene nitrosite	N (63°)[e]	+ 325	640	625	165
		+ 295	625		
Caryophyllene nitrosite	A	+ 6,200	623	680	164
		− 1,830	691		
Dinitrocamphane	A	− 5,930	302	280	166
		+ 2,960	267		
Xanthates and Related Compounds					
(+)-Bornyl methyl xanthate (XV)	HC	− 1,200	376	354	158
		+ 2,260	345		
(+)-Bornyl ethyl xanthate	HC	− 1,140	380	358	158
		+ 1,400	352		
(−)-Menthyl methyl xanthate (XVI)	HC	+ 1,620	378	353	158
		− *5,670*	341		
Methylene di-(+)-bornyl xanthate	HC	− 2,320	382	355	158
		+ *1,540*	351		
Methylene di-(−)-menthyl xanthate	HC	+ 4,320	380	355	158
		− *5,420*	344		
(+)-Bornyl dixanthogenide	HC	− 2,230	390	366	158
		+ *4,950*	355		
(−)-Menthyl dixanthogenide	HC	+ 1,880	390	363	158
		− *2,030*	344		
(+)-Bornyl diphenyl dithiourethane	HC	+ 1,680	578	520	158
		− *6,730*	436		
(−)-Menthyl diphenyl dithiourethane	HC	− 2,430	570	515	158
		+ *6,180*	436		

[a] Solvents: A, alcohol; C, chloroform; E, ether; HC, hydrocarbon (hexane, heptane, or cyclohexane); N, none (i.e., pure liquid).

[b] Epimeric with santonide at C-11.

[c] Negative cotton effect superimposed on plain negative curve.

[d] Multiple cotton effect superimposed on negative plain curve.

[e] Smooth curve at 14°.

more detail in Table II and discussed briefly on pages 261–262. As Kuhn has pointed out repeatedly, attempts to determine the character

of an absorption band from measurements at wavelengths remote from it are dangerous. Kuhn says (e.g., 138) "The optical activity of an absorption band is only understood when the rotation has actually been measured in the region of absorption itself." (See also Balfe, 40.)

(*a*) *Halogen Derivatives.* The dispersion curves of halogen compounds of the type R·CHX·Me show steeply rising or falling curves in the region of absorption (220, 240, 280 mμ for Cl, Br, I, respectively) but no maximum or minimum.

Esters of α-halogen substituted acids (R·CHX·CO_2Me; X = Cl, Br) on the other hand show one pronounced maximum or minimum at about 250–260 mμ. This region is at present rather inaccessible, but it may well be that in future compounds of this type will be useful for structural purposes.

(*b*) *Azides and Azido Acids.* The behavior of these compounds parallels roughly that of the halides and halogen-substituted acids. In the simple azide (Et·CHN_3Me) the absorption band at 280–290 mμ is apparently inactive and the dispersion curve is plain. Derivative of the acid (Me·CHN_3·CO_2H) give characteristic waves.

(*c*) *Phenyl-Substituted Carbinols and Acids.* α-Phenylpropionic acid (Ph·CHMe·CO_2H) and alkylphenyl carbinols (Ph·CHOH·R) show plain curves, even in the region about 250 mμ where absorption occurs.

Derivatives of mandelic acid (Ph·CHOH·CO_2H) and atrolactic acid (Ph·CMeOH·CO_2H), however, show much steeper plain curves (so far as can be measured) or in many cases a definite maximum.

There are strange anomalies, however, even among the few compounds studied; while Ph·CHOMe·$CONMe_2$ shows a positive Cotton effect curve with a peak at ~250 mμ, the curve of the corresponding methyl ester is negative. Kuhn (138) points out that this is an example where the limits of validity of the "vicinal rule" are overstepped. In the atrolactic acid series, both methyl ester and dimethylamide give positive Cotton effect curves.

The recent work of Lyle (159a) includes some compounds of the general type Ar·$CH(NH)_2$·R which show Cotton effects and some which do *not.*

(*d*) *Carbonyl Compounds.* Since the greater part of this chapter is devoted to recent work on carbonyl compounds, little need be said regarding the few classical measurements in this group.

Several branched-chain aldehydes of type $R_1R_2CH·(CH_2)_n·CHO$ were studied by Levene and Rothen (147) and some of them gave typi-

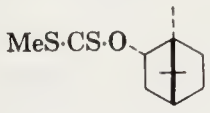
cal carbonyl "waves." The aldehyde forms of some monosaccharide polyacetates were shown by Lowry *et al.* (112) to give similar curves.

The one group of ketones studied in any detail in the early work consists of camphor and its derivatives.

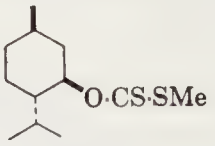
Djerassi and Geller (24) studied series of aliphatic aldehydes and ketones ($EtMeCH \cdot (CH_2)_n \cdot CHO$ and $EtMeCH \cdot (CH_2)_n \cdot CO \cdot CH_3$) in which the distance between the carbonyl group and the asymmetric center was varied.

(*e*) *Nitrites.* Nitrites of three related carbinols ($R \cdot CHONO \cdot CH_3$; R = *n*-C_6H_{13}, cyclohexyl, and Ph) were studied very thoroughly by Kuhn and his collaborators (138). These compounds, which absorb at ~365 mμ, provide the best examples in the classical work of multiple Cotton effect curves. Kuhn points out that a complete understanding of such curves requires a study of the circular dichroism of the compounds (difference in refractive index for right- and left-handed circularly polarized light).

(*f*) *Xanthates and Related Compounds.* Tschugaeff (194) and subsequently Lowry (158) made extensive studies of xanthates ($RO \cdot CS \cdot SMe$) and related derivatives of thiocarbonic acid containing asymmetric terpene residues such as bornyl and methyl (XV, XVI).



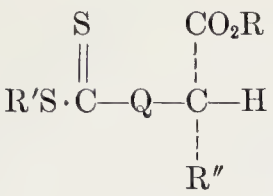
(XV)
(+)-Bornyl
methyl xanthate



(XVI)
(−)-Menthyl
methyl xanthate

Tschugaeff's work was restricted to the region above 460 mμ and therefore did not cover any absorption bands (except for a few dithiourethanes of the type $RO \cdot CS \cdot NPh \cdot CSPh$). Lowry and Hudson (158), however, were able to take measurements down to about 340 mμ and therefore to cover in large part the absorption bands at ~360 mμ. The curves were typical single Cotton effect curves; Lowry and Hudson compared their experimental curves with those calculated according to various theoretical equations.

Recently Sjöberg, Fredga, and Djerassi (31) have shown the value of the dispersion curves of xanthates and dithiocarbamates (XVIa; Q = O or NH) in correlating the stereochemistry of α-hydroxy- and α-amino acids.



(XVIa)

C. RECENT WORK ON ALICYCLIC KETONES AND RELATED COMPOUNDS

1. General

It has already been stated that the only connected program of work with a photoelectric spectropolarimeter has been that carried out by Djerassi and his colleagues (1–31). Starting from a study of some steroid hormones and related simpler steroids, Djerassi soon found the following working rules.

1. Among the types of compounds readily available in the alicyclic series, saturated ketones give the most useful curves for structural and stereochemical purposes; their weak absorption maxima at 290 mμ

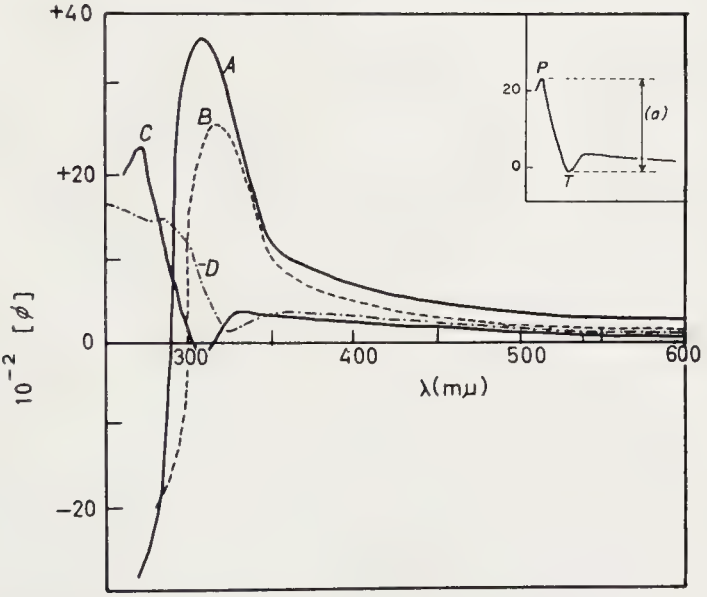


Fig. 4. Saturated ketones. Single Cotton effect curves, illustrating differences due to stereochemistry and to solvents. *A*, 5*α*-Cholestan-3-one (XXVI) in methanol; *B*, the same compound in dioxane; *C*, methyl-3-oxo-5*β*-cholanate (XXVII) in methanol; *D*, lanostan-3-one (LVII) in methanol. Inset shows peak (*P*), trough (*T*), and amplitude (*a*).

give rise to typical single Cotton-effect curves (Figs. 4 and 5) at about the same wavelength.

2. α,β-Unsaturated ketones also give useful curves, though these have more complex forms, the multiple Cotton effects over the range 380–300 mμ being associated with the weak absorption maximum at 320–350 mμ.

3. The ketone group is the only one* where it is generally possible to measure the dispersion curve right through the optical active absorption band. Other absorbing groups are either not optically active or else so strongly absorbing that the rotation cannot be measured near the absorption maximum, and the Cotton effect cannot therefore be studied.

4. Compounds containing no function absorbing light above about 260 mμ (e.g., hydrocarbons, alcohols, esters, acids) give dispersion curves showing no dramatic features; these are of much less value for structural purposes than the Cotton effect curves.

While these "plain curves" have no dramatic features, their positive or negative drift toward lower wavelengths may be sufficient to distort the Cotton effect curves. In interpreting dispersion curves of ketones, it is always desirable to know the background curve of the corresponding hydrocarbon or alcohol, which may be subtracted from that of the ketone if necessary. For an example see Section V-A.

5. Extension of the generalized method of molecular rotation differences to dispersion curves. (*a*) Enantiomorphs have dispersion curves which are *necessarily* mirror images of one another. (*b*) Compounds which are of the same stereochemical type in the neighborhood of the carbonyl group have dispersion curves of the same sign. (This is an extension of the generalized method of molecular rotation differences used by Klyne (126,127,133) to correlate steroids, triterpenes, diterpenes, etc.) (*c*) Compounds which are of enantiomorphic character in the neighborhood of the carbonyl group have dispersion curves of *antipodal* types, i.e., curves which are approximately mirror images of one another. (For examples see Section IV-D and Fig. 5).

The following sections of the text with tables and figures present a selection of the data collected by Djerassi's school over the last five years. The compounds considered included most of the simpler ref-

* Professor C. Djerassi (personal communication) has examined a number of aliphatic nitro compounds. The low intensity absorption band at about 280 mμ gives useful dispersion curves. See also ref. 31.

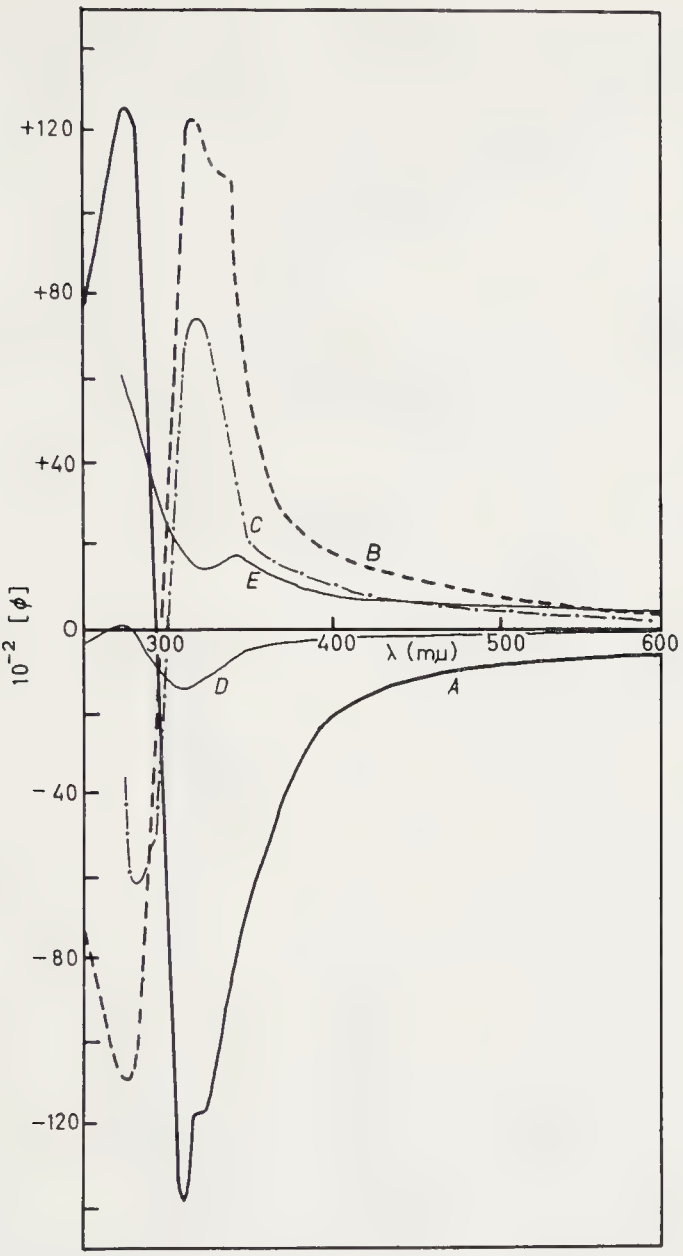
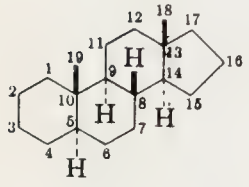


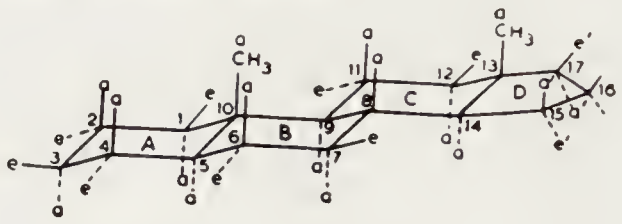
Fig. 5. Saturated ketones (in methanol unless otherwise stated). Single Cotton effect curves. *A*, 3β-Hydroxy-5α-androstan-16-one (XLII); *B*, 2-oxo-5α-*A*-norcholestane (XLIII); *C*, 5α-androstan-17-one (XXVIII) in dioxane; *D*, 3β-acetoxy-5α-cholestan-7-one; *E*, 5α-cholestan-1-one (XXIV). Curves *A* and *B* illustrate enantiomeric types; curves *C* and *D*, differences in amplitude due to position of keto group; and curve *E*, an abnormal ketone curve.

erence substances including steroids with one carbonyl group and normal all-*trans* stereochemistry. Other more difficult examples including conformational problems are introduced as required for the discussion in Section IV.

TABLE III
General Formulas of Steroids

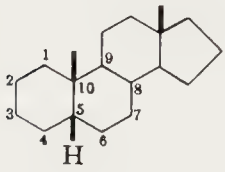


(XVIIa)

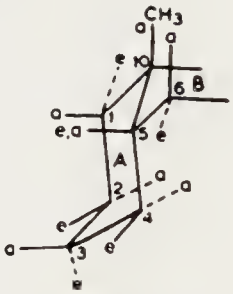


(XVIIb)

5α-Steroid



(XVIIIa)



(XVIIIb)

5β-Steroid

No. of C atoms	Side chain at C-17 (R)	Name[a] (IUPAC rules)	Old name	
			5α (XVII)	5β (XVIII)
19	None	Androstane	Androstane	Aetiocholane
21	$\cdot CH_2CH_3$	Pregnane	Allopregnane	Pregnane
24	$\cdot CHMe \cdot CH_2CH_2CH_3$	Cholane	Allocholane	Cholane
27	$\cdot CHMe \cdot CH_2CH_2 \cdot CHMe_2$	Cholestane	Cholestane	Coprostane
20	$\cdot CO_2H$	Etianic acid	Alloaetiocholanic acid	Aetiocholanic acid
22	$\cdot CHMe \cdot CO_2H$	Dinorcholanic acid	Bisnorallocholanic acid	Bisnorcholanic acid
23	$\cdot CHMe \cdot CH_2 \cdot CO_2H$	Norcholanic acid	Norallocholanic acid	Norcholanic acid
24	$\cdot CHMe \cdot CH_2 \cdot CH_2 \cdot CO_2H$	Cholanic acid	Allocholanic acid	Cholanic acid

[a] Always to have 5α or 5β prefix, except when compound has 4,5 or 5,6 double bond.

2. *Nonabsorbing Compounds: Plain Curves*

Nonabsorbing compounds show plain dispersion curves with no distinctive features, the magnitude of the rotation (positive or negative) becoming greater as one passes to lower wavelengths. A few representative curves are shown in Figure 1 (p. 245), and data are listed in Table IV.

TABLE IV
Plain Curves

Compound	[ϕ] 589 mμ	[ϕ] ~300 mμ[a]		Ref.[b]
Saturated Hydrocarbon (in Dioxane)				
5α-Androstane	+ 2	+ 30	(330)	1.V
Unsaturated Hydrocarbons (in Dioxane)				
5α-Cholest-1-ene	+ 75	+ 530	(300)	5.XI
5α-Cholest-2-ene	+250	+1650	(290)	5.XII
5α-Cholest-3-ene	+180	+1520	(300)	5.XIII
Cholest-4-ene	+270	+1760	(300)	5.XIV
Cholest-5-ene	−240	−1200	(300)	5.XV
5α-Cholest-6-ene	−340	−2960	(290)	5.XVI
Alcohols and Their Esters (in Dioxane)				
5α-Cholestan-3β-ol	+ 92	+ 520	(290)	3.I
5α-Cholestan-3β-ol acetate	+ 55	+ 350	(290)	3.II
Cholest-5-en-3β-ol	−150	− 970	(290)	3.V
5α-Cholest-6-en-3β-ol acetate	−380	−2760	(300)	3.VI
5α-Cholest-7-en-3β-ol acetate	+ 35	− 210	(290)	3.IV
5α-Cholest-8(14)en-3β-ol acetate	+ 40	+ 75	(320)	3.III
Phenol (in Dioxane)				
Estradiol-17β (XIX)	+270	+1200	(315)	2.IV
Carboxylic Acids and Their Esters (in Methanol)[c]				
5β-Cholanic acid (m.e.)	+130	+ 890	(260)	6.I
3α-Hydroxy-5β-cholanic acid (m.e.)	+ 60	+ 940	(280)	unp.
3α-Acetoxy-5β-cholanic acid (m.e.)	+108	+1250	(260)	unp.
3α,12α-Dihydroxy-5β-cholanic acid	+200	+1850	(250)	6.II
3α,12α-Dihydroxy-5β-norcholanic acid	+200	+1250	(250)	6.III
3α,12α-Dihydroxy-5β-dinorcholanic acid	+110	+ 650	(255)	6.IV
3α,12α-Dihydroxy-5β-etianic acid	+300	+4740	(250)	6.V
3β-Hydroxy-5α-etianic acid (m.e.)	+170	+3780	(250)	6.VI

(*continued*)

TABLE IV (*continued*)

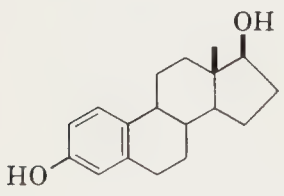
Compound	[ϕ] 589 mμ	[ϕ] ~300 mμ[a]		Ref.[b]
Spirostans (in Dioxane)				
5α,22*a*,25*D*-Spirostan	−320	−1440	(290)	4.I
5α,22*a*,25*D*-Spirostan-3β-ol	−240	−1030	(300)	4.II
22*a*,25*D*-Spirost-5-en-3β-ol	−470	−2300	(300)	4.VI
5β,20α,22*a*,25*D*-Spirostan-3β-ol (smilagenin) (XX)	−290	−1200	(300)	4.X
5β,20α,22*a*,25*L*-Spirostan-3β-ol (sarsasapogenin)	−340	−1460	(300)	4.Xa
5β,20β,22*a*,25*D*-Spirostan-3β-ol (cyclo-ψ-smilagenin)	−260	−1270	(300)	4.–
5β,20β,22*b*,25*L*-Spirostan-3β-ol (cyclo-ψ-sarsasapogenin)	+150	+ 815	(300)	4.Xb
Lactones (in Dioxane)				
3β,14β-Dihydroxy-5β-cardenolide (as XXI)	+ 4	+1690	(275)	15.I
3β,14β-Dihydroxy-5β-bufadienolide (as XXII)	− 80	−4500	(320)	15.IV

[a] Values in parentheses are the wavelengths in mμ for the rotation given; these are usually the lowest wavelengths measured.

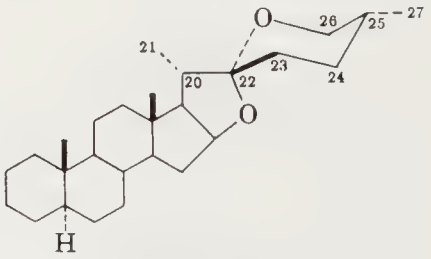
[b] Reference numbers (e.g., 1.V) indicate, first, the number of the paper in Djerassi's series and, second (in Roman figures), the number allotted to the compound in that paper; unp. = unpublished.

[c] m.e. = methyl ester.

Generally speaking the rotation values at about 300 mμ are anything from 5 to 10 times as large as those for the sodium D line (589 mμ); for etianic acid derivatives the factor is as high as 20 times. Unsaturated lactones (of both cardenolide and bufadienolide types) show

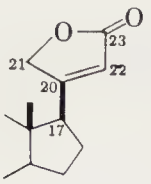


(XIX)
Estradiol-17β

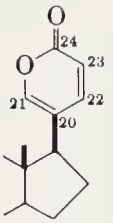


(XX)
5α-Spirostan (20α,22*a*,25*D*)

much steeper curves ($[M]_{300}$ is 50 times $[M]_{589}$, or more); in both these classes of compound the lower limit of dispersion measurements is approaching an absorption band.



(XXI)
Cardenolide
side chain



(XXII)
Bufadienolide
side chain

After much effort Djerassi (3) decided that the labor involved in trying to fit one- or two-term Drude equations to these curves was not worth while. For the purpose of empirical correlations, extensive studies on series of isomers among nonabsorbing compounds will be necessary to indicate the most convenient features for structural comparisons: these may be the ratio of rotations at two standard wavelengths (say $[\phi]_{300}/[\phi]_{589}$), or $[\phi]_{300}$ itself (instead of $[\phi]_{539}$, or perhaps the slope and intercept of the curve obtained when $1/[\phi]$ is plotted against λ^2.

For a discussion with many further examples of nonketonic steroids, see Jones and Klyne (116).

3. Saturated Ketones

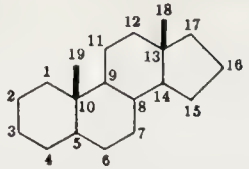
(*a*) *Single Cotton Effect Curves: General.* All the saturated ketones studied (with very few exceptions) show curves with one peak and one trough in the region 320–270 mμ, the midpoint of the "wave" being fairly close to the absorption maximum of the carbonyl band at about 290 mμ. The differences between the curves for individual ketones are presumably due to minor differences in the asymmetric environment around the carbonyl group.

Some representative curves are shown in Figures 4 and 5, data for the peaks and troughs of steroid ketones and bicyclic analogs in Tables V and VI. Many of the potential uses of dispersion curves are apparent from a study of these figures and tables. (The extraordinary behavior of 1-oxo-5α-steroids and 17*a*-oxo-*D*-homosteroids, which give no real peaks and troughs, is discussed in Section V-C.)

TABLE V

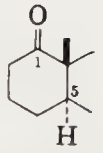
Steroid Ketones

(Values in methanol)



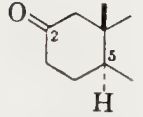
(XXIII)

Steroid numbering



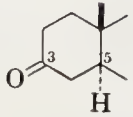
(XXIV)

5α-1-one



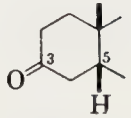
(XXV)

5α-2-one



(XXVI)

5α-3-one

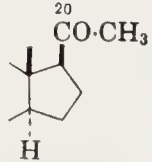


(XXVII)

5β-3-one

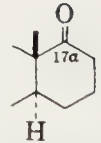
(XXVIII)

17-one



(XXIX)

20-one



(XXX)

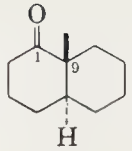
D-Homo-17*a*-one

Compound	[φ] and λ			Sign and amplitude of wave, $10^{-2}a$	Ref.[b]
	589 mμ	Peak[a]	Trough[a]		
	5α-Series				
5α-Cholestan-1-one (XXIV)	+440	+ 1,560 (338)	+ 1,440 (318)[c]	—	5.I
5α-Cholestan-2-one (XXV)	+230	+ 6,300 (310)	− 5,850 (267)	+121	5.II
5α-Cholestan-3-one (XXVI)	+210	+ 3,700 (307)	− 2,850 (267)	+ 65	5.III

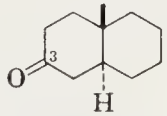
5α-Cholestan-4-one	+ 50	− 3,000 (307)	+ 6,380 (267)	− 94	5.IV
5α-Cholestan-6-one, 3β-acetoxy-	− 74	− 3,550 (306)	+ 4,040 (270)	− 76	5.VI
5α-Cholestan-7-one, 3β-acetoxy-	−160	− 1,490 (310)	+ 65 (274)	− 16	5.VII
5α-Ergostan-11-one, 3β-acetoxy-	+147	+ 1,300 (322)	+ 130 (282)	+ 12	14.V
		5β-Series			
5β-Androstan-1-one, 17β-methoxycarbonyl	−100	− 5,570 (318)	+ 8,000 (285)	−136	15.XI
5β-Cholestan-3-one (XXVII)	+190	− 80 (307)	+ 2,600 (265)	− 27	6.X
5β-Cholestan-4-one	+180	+ 1,230 (300)	+ 930 (278)	+ 3	7.LXIV
5β-Cholestan-6-one	− 19	− 3,020 (308)	+ 4,700 (270)	− 77	6.XVI
5β-Cholan-7-one, 3α-hydroxy-, -24-oic acid	−100	+ 360 (312)	− 2,540 (264)	+ 29	6.XIX
		5α- and 5β-Series			
5β-Cholan-12-one, 3α-acetoxy-, -24-oic acid (methyl ester)	+450	+ 3,330 (305)	+ 2,380 (272)	+ 10	6.XXVI
5α-Cholestan-15-one, 3β-hydroxy-	+200	+ 7,180 (316)	− 7,400 (275)	+146	5.VIII
5β-Cholestan-16-one	−440	−13,300 (317)	+14,600 (276)	−279	5.IX
5α-Androstan-17-one (XXVIII)[d]	+220	+ 7,500 (318)	− 6,500 (279)	+140	7.XV
5α-Pregnan-20-one, 3β-acetoxy- (XXIX)	+320	+ 8,850 (308)	− 8,780 (262)	+178	15.—
	+260[d]	+ 7,300 (315)	− 6,070 (275)	+134	
		D-Homo Series			
5α-*D*-Homoandrostan-17*a*-one, 3β-hydroxy- (XXX)	−114	− 580 (320)	− 500 (312)[c]		5.X
5α-*D*-Homoandrostan-17-one, 3β-hydroxy-17*aβ*-methyl-[d]	−170	− 5,480 (315)	+ 4,150 (275)	− 96	7.XXXV

[a] Values in parentheses are wavelengths (λ) in mμ.
[b] Djerassi *et al.;* see Table IV.
[c] Not a true "wave."
[d] In dioxane.

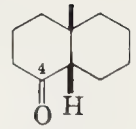
TABLE VI
Bicyclic Ketones
(Values in methanol except as noted)



(XXXI)
trans-1-Oxo

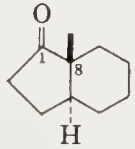


(XXXII)
trans-3-Oxo

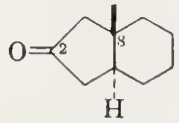


(XXXIII)
cis-4-Oxo

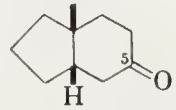
9-Methyldecalones



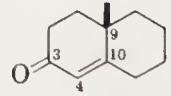
(XXXIV)
trans-1-Oxo



(XXXV)
trans-2-Oxo



(XXXVI)
cis-5-Oxo



(XXXVII)
9-Methyl-Δ^4-octalone-3

8-Methylhexahydroindanones

Compound	[ϕ] and λ Peak[a]	[ϕ] and λ Trough[a]	Sign and amplitude of wave, $10^{-2}a$	Ref.[b]
	Saturated Ketones			
9-Methyldecalin derivatives				
trans-1-Oxo (XXXI)[c]	+ 2,340 (318)	− 840 (277)	+ 32	7.III
trans-3-Oxo (XXXII)[c]	+ 2,240 (318)	− 2,460 (275)	+ 47	E.7.IX

trans-4-Oxo	− 1,340[d] (305)	+ 1,900[d] (265)	− 32[d]	E.16.XI
cis-1-Oxo	+ 2,240 (312)	− 2,820 (275)	+ 51	16.XVIII
cis-3-Oxo	− 345 (308)	+ 735 (268)	− 11	E.16.VII
cis-4-Oxo (XXXIII)	+ 615 (322)	− 500 (275)	+ 11	E.16.X
4,4,9-Trimethyldecalin derivative				
trans-3-Oxo	− 850 (320)	+ 260 (275)	− 11	E.16.XXII
8-Methylhexahydroindane derivatives				
trans-1-Oxo (XXXIV)[c]	− 4,730 (322)	+ 4,110 (280)	− 88	7.VII
trans-2-Oxo (XXXV)	+11,300 (315)	−10,600 (275)	+219	E.7.XI
trans-5-Oxo	+ 2,150 (310)	− 1,950 (272)	+ 41	E.19.X
cis-5-Oxo (XXXVI)[c]	− 290 (316)	+ *670* (280)	− *10*	7.XIII[e]
trans-4-Oxo[f]	− 5,500 (305)	+10,000 (270)	−155	19.XIV
	Unsaturated Ketones			
9-Methyl-octalin derivatives				
trans-Δ^1-3-Oxo[c]	− 1,670 (380)	+ 2,300 (322)	− 40	E.16.V
Δ^4-3-Oxo (XXXVII)[c]	+ 960 (362)	+ 3,400 (308)	− 24	E.16.VI
$\Delta^{5(10)}$-4-Oxo[c]	+ 150 (362)	− 3,080 (312)	− 32	E.16.VIII

[a] Values in parentheses are wavelengths in mμ.

[b] Djerassi *et al.;* see Table IV.

E indicates that the signs for rotation values given here are for the *enantiomer* of the compound actually measured. This is done so that all the ketones described here are those with the carbonyl group in the left-hand ring.

Additional Compounds: For *cis*- and *trans*-2-oxo-9-methyldecalins carrying additional methyl substituents, see refs. 85,202.

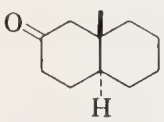
[c] In dioxane.

[d] Estimated from the equilibrium mixture of *cis* and *trans*.

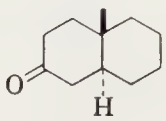
[e] See ref. 67b.

[f] With iso octyl substituent at C-1.

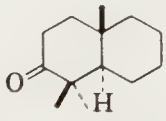
The characteristics of the curves which are of structural significance are (*a*) the *amplitude* (i.e., the difference in [ϕ] between peak and trough) of the wave and (*b*) the exact *wavelength* of peak, trough, or midpoint of the wave; the latter characteristic is usually considered



(XXXVIII)
"Me-near"



(XXXIX)
"Me-far"



(XL)
Lanostane type

9-Methyldecalone types

TABLE VII
Saturated Ketones: Summary

A. Wavelengths of "First Extrema" in Dispersion Curves Illustrating Constitutional and Solvent Effects

Type	λ (dispersion), mμ		λ_{max} (absorption), mμ	
	Dioxane	Methanol	Dioxane	Methanol or ethanol
Cyclohexanones				
Standard value (for bicyclic, steroid, and other series)	312–320	305–312[a]	284–292	284–296[e]
3-Oxo-4,4-dimethyl compounds (lanostane series)	325–330	315–325[b]		
11-Oxosteroids	325–328	318–325		298
Cyclopentanones				
Standard value (for bicyclic, steroid, and other series)	320–325	312–318[c]	294–297	290–303
Miscellaneous				
Aldehydes (19-oxosteroids)	327–337[d]			
20-Oxosteroids	312–315	308		

[a] 1-Oxo-5β-steroid at 320 mμ; see text regarding 4-oxo-5β-steroid and its bicyclic analog.

[b] Δ^8-Compounds at 300–305 mμ.

[c] Includes bridged-ring compounds of type LXIX.

[d] One value at 322 mμ.

[e] 17*a*-Oxo-*D*-homosteroid has λ (absorption) 275–277 mμ in methanol; dispersion very unusual (see text).

B. Amplitudes of "Waves," Illustrating Constitutional Effects[f]

Class	Subclass[g]	No. of examples	Amplitude, $10^{-2}a$	
			Range	Mean
trans-α-Hexahydroindanones	—	4	66–144	110
trans-β-Hexahydroindanones	—	6	180–279	230
trans-α-Decalones	Me-near	3	14–32	21
trans-α-Decalones	Me-far	2	32, 93	—
trans-α-Decalones	HH	5	50–60	56
cis-α-Decalones	Me-near	3	50–136	133
cis-α-Decalones	Me-far	2	3, 11	—
trans-β-Decalones	Me-near	8	77–136	107
trans-β-Decalones	Me-far	18	29–70	48
trans-β-Decalones	Lan.	12	11–39	24
trans-β-Decalones	HH	2	63, 90	—
cis-β-Decalones	Me-far	12	10–46	25

[f] This table includes values in dioxane and in methanol; it does *not* include values for compounds in which abnormalities are to be expected (e.g., those due to axial methyl groups, cf. p. 320).

[g] The abbreviations indicate the substituents at the ring junctions between the carbonyl-carrying ring and the rest of the molecule as follows:

Me-near = angle methyl at ring junction near carbonyl (XXXVIII).

Me-far = angle methyl at ring junction far from carbonyl (XXXIX).

Lan. = lanostane type, like Me-far but with *gem*-dimethyl adjacent to carbonyl (XL).

HH = hydrogen at both ring junctions.

as a difference, Δ, from a standard curve, and attention is generally concentrated on the higher wavelength feature of the wave at about 300–320 mμ because of difficulty in measuring the "lower end." In all that is said here, the idea of enantiomeric types, which will have mirror image curves, is implicit.

Data for a more extensive series of saturated ketones are summarized in Table VII and set out in Tables VIII and X. These are mainly bicyclic ketones, and polycyclic compounds with a ketone group in a terminal ring. The following structural and stereochemical features are of importance in determining the position and shape of the Cotton effect curve: (*1*) ring size (cyclopentanone or cyclohexanone), (*2*) ring fusion (*cis* or *trans*), (*3*) relation of carbonyl to ring junction (α- or β-decalone or hexahydroindanone), (*4*) substituents at ring junctions (hydrogen or methyl), (*5*) presence or absence of substituents α to carbonyl (halogen, hydroxyl, alkyl), (*6*) conformation of these

TABLE VIII
Cyclopentanones

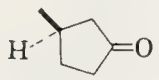
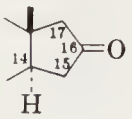
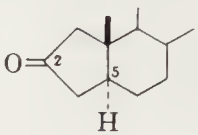
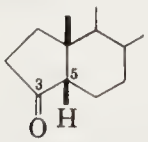
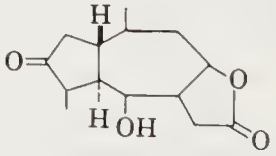
Compound	$10^{-2}a$[a]	Notes	Ref.[b]
Monocyclic Ketone			
3-Methylcyclopentanone (XLI)	+86		22.III
 (XLI) 3-Methylcyclopentanone	 (XLII) 16-Oxosteroid		 (XLIII) 2-Oxo-5α-*A*-norsteroid
Bicyclic and Polycyclic Ketones			
I. *trans*-α-Hexahydroindanones			
8-Methyl-*trans*-hexahydroindan-1-one	−88 D		7.VII
17-Oxosteroid (XXXVIII)	E −140 D	Fusion with ring B increases amplitude (cf. pp. 330–332)	7.XV
14α-Methyl-17-oxosteroid	E −66 M	Lanane series; *a* smaller than above	17.LXXIII
15-Oxosteroid	E −144 M	CO adjacent to H at ring junction	5.VIII
II. *cis*-α-Hexahydroindanones			
17-Oxo-14β-steroid	E −34 M	Amplitude much smaller than *trans* isomer	7.XVI
3-Oxo-*A*-nor-5β-steroid (XLIV)	+133 M	CO adjacent to H at ring junction	7.XIVb
III. *trans*-β-Hexahydroindanones (A. With one methyl and one hydrogen at ring junctions)			
8-Methyl-*trans*-hexahydroindan-2-one (XXXV)	+219 +206!		E7.XI 19.VII
2-Oxo-*A*-nor-5α-steroid (XLIII)	+234		7.XX
16-Oxosteroid (XLII)	E +264 E +279		7.XVIII 5.IX
 (XLIV) 3-Oxo-*A*-nor-5β-steroid		 (XLV) 3-Oxoguaianolide type	

TABLE VIII (*continued*)

Compound	$10^{-2}a$[a]	Notes	Ref.[b]
(B. With two methyl groups at ring junctions)			
14α-Methyl-16-oxo-steroid	E +180 D	May be influenced by 7,9 (11) diene grouping	17.LXXV
(C. With two hydrogens at ring junctions)			
3-Oxoguaianolides	+183, 103!, 132! D		13.III, Va, Vb
(XLV)[c]	+136!, 55!, 88! D		13.IX, X, XI
	+146 M		13.XIX
IV. *cis*-β-Hexahydroindanone			
2-Oxo-*A*-nor-5β-steroid	−152 M		7.XIVa

[a] Values are given for the signs and amplitudes ($10^{-2}a$) of the "waves" at approximately 300 mμ. E indicates enantiomer (see p. 251). Solvents are indicated by D (dioxane) and M (methanol). An exclamation mark (!) indicates that the extremum of lower wavelength was not reached; the amplitude may therefore be *greater* than the value given.

[b] Djerassi *et al.*, see Table IV.

[c] These compounds are not strictly α-hexahydroindanones, since the cyclopentanone ring is fused to a cycloheptane ring; this can reasonably be considered as *roughly* equivalent to a cyclohexane ring.

α-substituents (axial or equatorial), (*f*) abnormal ring conformations or distortions (may be conditioned by *e* and *f* above).

A smaller number of monocyclic ketones, acyclic (or side chain) ketones, ketones in the middle rings of polycyclic structures, and aldehydes are also considered.

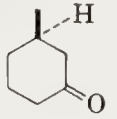
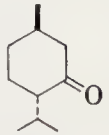
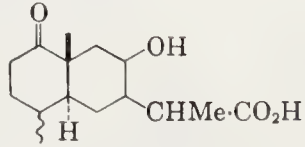
Djerassi and Krakower (21) have investigated the effect of ring size on the dispersion curves of 3-methylcycloalkanones.

The effects of factors (*a*)–(*d*), which might be called the general stereochemistry of the compound, are illustrated by Tables VIII and IX. For the sake of simplicity in comparisons, the signs for ketone "waves" shown in these tables are all for left-hand terminal rings (like the A ring of the steroid nucleus, cf. XXXVIII–XL, XLIII–XLIV). Where the compound measured was of enantiomeric type, e.g., a 16-oxosteroid (XLII; actually −264), the sign is changed and the value prefixed with the letter E thus (E +264) for ready comparison with the *A*-nor-2-oxo compound (XLIII, +234).

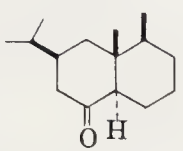
The symbol ! (exclamation mark) against an amplitude indicates (except in Table XIII) that the peak or trough of lower wavelength

TABLE IX

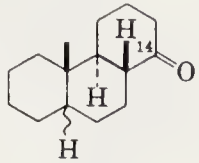
Cyclohexanones

Compound	$10^{-2}a^{a}$	Notes	Ref.[b]
	Monocyclic Ketones		
3-Methylcyclohexanone (XLVI)	+26 M +29 HC		11.22.I 97
2,4-Dimethylcyclohexanone (*cis*) (from actidione)	−2 M		92
3-Methyl-6-isopropylhexanone (*trans*) (XLVII)	+10 D	Menthone	8.I
 (XLVI) 3-Methylcyclohexanone	 (XLVII) Menthone	 (XLVIII) 1-Oxo-7-hydroxysantanic acid	
	Bicyclic and Polycyclic Ketones		
I. *trans*-α-Decalones (A. With methyl group at ring junction adjacent to carbonyl)			
9-Methyl-*trans*-decalone-1	+32 D		7.III
2,5,5,9-Tetramethyl-decalone-1	+14! M		E17.LXXI
9-Methyl-2X-decalone-1[c]	+62 M	9,11-*sec*-Steroid	E17.LXXII
1-Oxo-7-hydroxysantanic acid (XLVIII)	+17 D		7.LXIX
1-Oxo-5α-steroid (XXIV)	—	Grossly abnormal; no "wave" (see Table V); note effect of additional methyl group	5.I
17*a*-Oxo-*D*-homosteroid (XXX)	—		5.X
17α-Methyl-17*a*-oxo-*D*-homosteroid	−30		Unp.

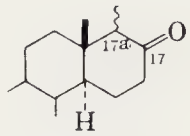
(B. With methyl group at ring junction remote from carbonyl)			
9-Methyl-*trans*-decalone-4	−32 M	Approx.; calc. from equilibrium mixture	E16.XI
8,9-Dimethyl-2-isopropyl-*trans*-decalone-4 (XLIX)	+109 M	*trans*-Tetrahydroeremophilone	7.LXI*
4-Oxo-5α-steroid	−93 M		5.IV
(C. With hydrogens at both ring junctions)			
Des-*D*-14-oxosteroids (L)	−60, −59, −56 M		9.V–VII
	−50, −55 D		9.III–IV



(XLIX)
trans-Tetrahydroeremophilone



(L)
14-Oxo-des-*D*-steroid



(LI)
17-Oxo-*D*-homosteroid

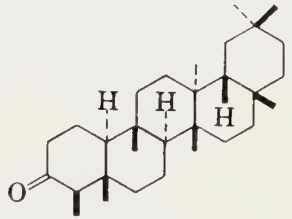
II. *cis*-α-Decalones (A. With methyl group at ring junction adjacent to carbonyl)			
9-Methyl-*cis*-decalone-1	+51 M		15.XII
1-Oxo-5β-steroid	−136, −111 M		15.XI
(B. With methyl group at ring junction remote from carbonyl)			
9-Methyl-*cis*-decalone-4 (XXXIII)	+11 M		E16.X
8,9-Dimethyl-2-isopropyl-*cis*-decalone-4	+37 M		7.LXIa*
4-Oxo-5β-steroid	+3 M		7.LXIV
III. *trans*-β-Decalones (A. With angle methyl group at ring junction nearer to carbonyl group; "2-oxo-5α-steroid" type)			
2-Oxo-5α-steroid (XXV)	+121 M		5.II
	+77 D		14.XIX
17-Oxo-*D*-homosteroid, with 17$a\beta$-Me (LI)	E +96 D		7.XXXV

* The configurations shown here are not as in ref. 7, but as corrected by later work (202).

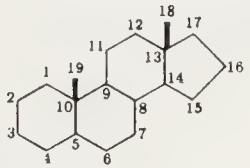
(*continued*)

TABLE IX (*continued*)

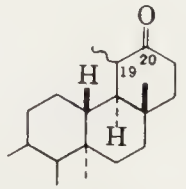
Compound	$10^{-2}a$[a]	Notes	Ref.[b]
17-Oxo-*D*-homosteroid, with 17*a*α-Me	E +21 D	Axial methyl at 17*a* reduces amplitude	7.XXXIV
Friedelin (LII)	E +110 D		7.XXXVI
1-X-5,5,9-Trimethyldecalones-2			
Eperuic acid derivative	+100! D	(X = $CH_2CH_2CHMe{\cdot}CO_2Me$)	12.Ib
Labdanolic acid derivative	E +80 D	(X as above)	12.IIIb
Manool derivative	E +136! D	(X = $CH_2CH_2CO_2H$)	12.IIb
Onocerin derivative	E +135! D	Value is for half molecule	12.IV



(LII)
Friedelin



(LIII)
Steroid numbering

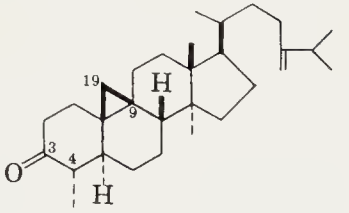


(LIV)
30-Nortaraxastan-20-ones

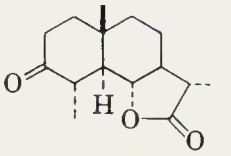
(B. With angle methyl group at ring junction remote from carbonyl; "3-oxo-5α-steroid type")

3-Oxo-5α-steroids(XXVI)	+65, +45 M +37, +46 D		5.III, 6.VII 4.XVII, 17.XXIII
Unsaturated			
3-Oxo-5α-steroid-7-ene	+56 M	Double bonds make little difference except for $\Delta^{8(14)}$	17.LXXXIX
3-Oxo-5α-steroid-8-ene	+48 M		17.XC
3-Oxo-5α-steroid-8(14)-ene	+72 M		17.XCI
3-Oxo-5α-steroid-14-ene	+55 M		17.XCII
3-Oxo-5α-steroids with additional alkyl groups			
2α-Me-3-oxo-5α-steroid	+62 M, +29 D		17.XXXV 17.XXXVII

2β-Me-3-oxo-5α-steroid	+73 M		17.XXXVI
4α-Me-3-oxo-5α-steroid	+54! M		17.XXIX
4β-Me-3-oxo-5α-steroid	+7 M		17.XXX
6α-Me-3-oxo-5α-steroid	+33 M		17.XXI
6β-Me-3-oxo-5α-steroid	+51 M		17.XXII
2,2-Me_2-3-oxo-5α-steroid	+80 M		17.XXXVIII
4α-Et-3-oxo-5α-steroid	+62 D		17.XXXI
30-Nortaraxastan-20-one (19β Me) (LIV)	+29 D		17.XXXIV
30-Nortaraxastan-20-one (19α Me)	+10 D		17.XXXIII
Cycloeucalenone (4α-methyl-3-oxo-9,19-cyclo structure) (LV)	+45 M		17.LXII
9-Methyl-*trans*-decalone-3 (XXXII)[d]	+47 D		E7.IX
5,9-Dimethyl-*trans*-decalone	+32 M		
3-Oxo-5α-santonanic acid	+70 M		17.XLV
3-Oxo-5α-santanolides (LVI)			
(4α-Methyl)	+33 M	"α"- and "γ"-Tetrahydrosantonins	7.XXXI
(4β-Methyl)	+20 M		7.XXXII
3-Oxo compound from cafestol (CXVII)	−49 D	Enantiomeric type to steroid	7.XLIV



(LV)
Cycloeucalenone

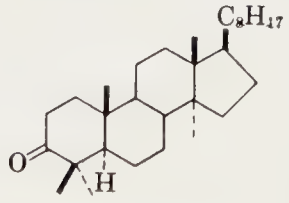


(LVI)
"α"-Tetrahydrosantonin

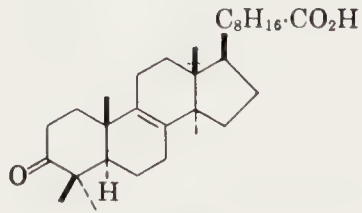
(*continued*)

TABLE IX (*continued*)

Compound	$10^{-2}\,a^{a}$	Notes	Ref.[b]
(C. As B above, but with *gem*-dimethyl group adjacent to carbonyl; "3-oxotriterpene type": 1. Lanostan-3-ones)			
Lanostan-3-one (LVII)	−16 M		17.LVIII
4,4-Dimethyl-5α-steroid-3-one	−11, −14, −12 D		17.XLVI–XLVIII
9,19-Cyclolanostan-3-ones	−39, −32 M	Cycloartenone and cyclolaudenone	17.LX–LXI
Lanost-7-en-3-one	−26 M −30! D		17.LXXIX
Lanost-7,9(11)-dien-3-one	−33 D		17.LXXXVI
Euph-7-en-3-one	−30! M	(13α,14β,17α) Dihydrobutyrospermone	17.LXXXIII
Euph-7-en-3-one; 20-epimer, with 24-en-26-oic acid	−27! M	Masticadienonic acid	17.LXXXIV
Lanost-8-en-3-one	+4 M	*Note reversal of sign*	17.LXXX
Lanost-8-en-3-one, with 24-methyl-21-oic acid (LVIII)	+49! M	Eburicoic acid deriv.	17.LXXXI

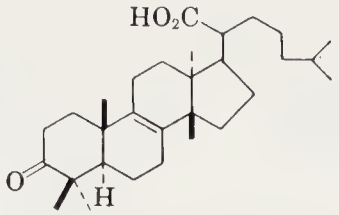


(LVII)
Lanostan-3-one



(LVIII)
24-Methyl-3-oxolanost-8-en-21-oic acid (from eburicoic acid)

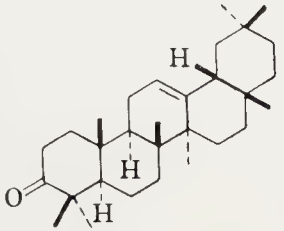
Tirucall-8-en-3-one, 21-oic acid (LIX)	+15! D	(13β,14β,17α,20-iso) Elemenonic acid	17.LXXXII
Tirucall-8-en-3-one, 24-en-26-oic acid	+28 M	Isomasticadienonic acid	17.LXXXV
4,4,9-Trimethyldecalone-3	−11 M		E16.XXII



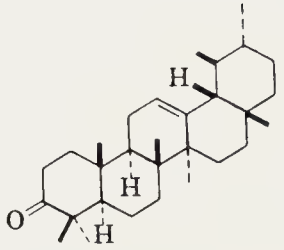
(LIX)
3-Oxotirucall-8-en-21-oic acid (clemenonic acid)

(2. Olean- and ursan-3-ones)

Olean-12-en-3-one (LX)	+12 M	25.XIX
Urs-12-en-3-one (LXI)	+8 M	25.XXXII



(LX)
Olean-12-en-3-one
("β"-amyrone)

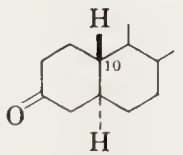


(LXI)
Urs-12-en-3-one
("α"-amyrone)

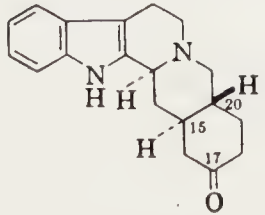
(*continued*)

TABLE IX (*continued*)

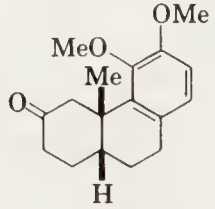
Compound	$10^{-2}a$[a]	Notes	Ref.[b]
Methyl 3-oxoolean-12-en-28-oate	+24 M		25.XX
28-Norolean-17-en-3-one (oleanone)	+41		25.XXV
(D. With two hydrogen atoms at ring junctions; "19-norsteroid type")			
3-Oxo-5α-19-norsteroid (LXII)	+90! M		17.LII
3-Oxo-15α-19-norsteroid, with methyl substituents			
1α-Me	+63 M		17.LIV
2,2-Me_2	+139 M		17.LVI
4,4-Me_2	−20, −15 M	Note reversal of sign, as in "ordinary" series with 19-methyl group	17.LV, LVII
Yohimbone (LXIII)	−106 D	Estimated from half wave	7.LII
IV. *cis*-β-Decalones (A. With angle methyl at ring junction near to carbonyl; "2-oxo-5β-steroid" type)			
Morphine degradation product (LXIV)	+60 M	Estimated from half wave	7.LIV
21-Oxotriterpene (machaerate) (LXV)	E +127 D		25.LVI



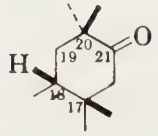
(LXII)
3-Oxo-19-nor-5α-steroid



(LXIII)
Yohimbone



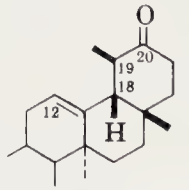
(LXIV)
Oxooctahydrophenanthrene (from morphine)



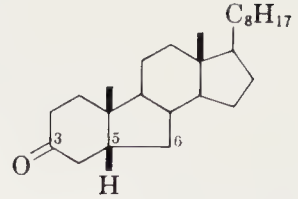
(LXV)
21-Oxooleanane derivative (from machaeric acid)

(B. With angle methyl at ring junction remote from carbonyl; "3-oxo-5β-steroid" type)

3-Oxo-5β-steroid (XXVII)[e]	−22, −34, −27 M −33, −40, −20 D	6.XI, —, X 15.VI, VIII, IX



(LXVI)
30-Norurs-
12-en-20-one



(LXVII)
B-Nor-5β-cholestan-
3-one

2β-Methyl-5β-steroid	−10 D		17.XL
4β-Methyl-5β-steroid	−22 M		17.XLI
9-Methyl-*cis*-decalone-3	−10 M		16.VII
9-Methyl-*cis*-decalone-3, with 8-CO_2Me	−10 M		16.XVII
3-Oxo-5β-santonanic acid	−46 D		17.XLIV
3-Oxo-5β-santanolide	−23 M	"β"-Tetrahydrosantonin	7.XXXIII
29,30-Dinorurs-12-en-20-one, 3β-benzoyloxy (LXVI)	−73 D		17.XLII
30-Norurs-12-en-20-one, 3β-benzoyloxy (LXVI)	−66 D		17.XLIII

[a] Values are given for signs and amplitudes ($10^{-2}a$) of the "waves" at approximately 300 mμ. E indicates enantiomer (see p. 251). Solvents are indicated by D (dioxane), M (methanol), and HC (hydrocarbon).

[b] Djerassi *et al.;* see Table IV.

[c] X = substituted cyclopentyl group.

[d] *B*-Nor analog of this +41 M (E19.X).

[e] *B*-Nor analog (LXVII) has $10^{-2}a$ = −55 M (19.XIII).

TABLE X
Miscellaneous Carbonyl Compounds

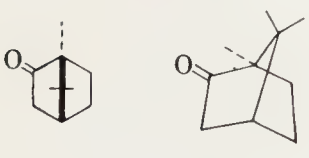
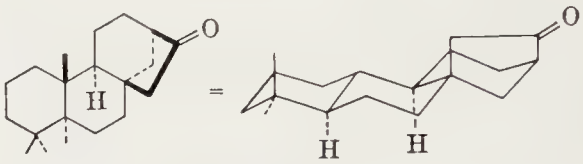
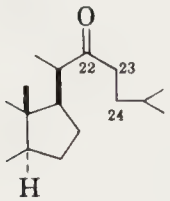
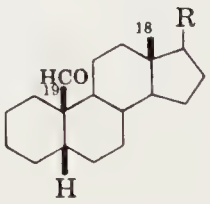
Compound	$10^{-2}a$[a]	Ref.[b]
Various Cyclic Ketones		
3-Methylcyclotetradecanone	−12 M	11.22.II
Camphor, Bicyclo-[2,2,1]-heptanone type (LXVIII)	+80 M +73 HC	14.—.157
 (LXVIII) Camphor		
 (LXIX) Bicyclo-[3,2,1]-octanone (from phyllocladene)		
Bicyclo-[3,2,1]-octanone type (LXIX)		
From phyllocladene	+108 D	7.XLII
From cafestol	+113 D	7.XLIII,62a, 101a
Enantiomeric type		
From steviol	−93 M	7.XLVII
Cuachichicine	−19 M	7.XLIX
From gibberellic acid	+79 M	76a,191a
Side Chain Ketones		
Pregnan-20-one (XXIX)	+178, 178 M +125, 134! D	15.—
Cholestan-22-one (LXX)	−30! M	Unp.
Cholestan-23-one	−52 M	Unp.
Cholestan-24-one	−14! M	Unp.
 (LXX) 22-Oxosteroid		
 (LXXI) 19-Oxosteroid		

TABLE X (*continued*)

Compound	$10^{-2}a$[a]	Ref.[b]
	Aldehydes	
Steroid 18-aldehyde[c] (from holarrhimine)	+64 D	15.XX
Steroid 19-aldehyde[c] (strophanthidin etc.)		
5β (LXXI)	−21, −35, −51 D	15.VI, XVII, XVIII
5α	+24 D	15.XIX

[a] Values are given for the signs and amplitudes ($10^{-2}a$) of the "waves" at approximately 300 mμ. Solvents are indicated by D (dioxane), M (methanol), and HC (hydrocarbon).

[b] Djerassi *et al.;* see Table IV.

[c] These values are probably subject to vicinal action.

has not been measured and that the true amplitude may be greater than that given here.

(*b*) *Wavelengths of "First Extrema": Constitutional Effects.* There are no very striking differences in the wavelengths in the "first extrema" for different classes of saturated ketones, except for those carrying α-halogen or α-hydroxyl substituents (see Sections III-C-4 and 5). The minor differences in λ (first turning point) due to constitutional effects are summarized in Table VII, part A; these differences in general run parallel with those of λ_{max} (absorption) as might be expected (cf. 1,17).

Attention may be drawn to an interesting difference between the 4-oxo-5β-steroid (λ, 300 mμ in methanol) and its bicyclic analog, *cis*-9-methyl-4-decalone (λ, 322 mμ in methanol). As pointed out by Djerassi and Marshall (16) these compounds probably differ in conformation (cf. Klyne, 129).

(*c*) *Amplitudes: Constitutional Effects.* The numerical values of the amplitudes for different types seem to fall into distinct ranges (Table VII, part B). The values for *trans*-β-hexahydroindanones, which are considerably greater than those for the *trans*-α-analogs, are noteworthy. A distinction which might be of considerable value for structural work, if it is confirmed by further values, is that between the two types of *trans*-β-decalones. Type XXXIX, which includes the 3-oxo-5α-steroids, has a hydrogen at the ring junction (C-5) nearest to the carbonyl group; type XXXVIII, which includes the 2-oxo-5α-steroids, has a methyl at the corresponding ring junction

(C-10), and shows a significantly larger amplitude. This is a distinction which would not, so far as the author is aware, be shown by any other physical technique. See also the Octant Rule (Section VI).

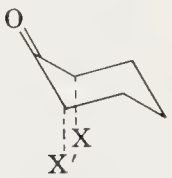
(*d*) *β-Effect.* It is appropriate here to emphasize the importance in relation to the sign of the Cotton effect of the stereochemistry of *atoms β to the carbonyl groups.* This is well illustrated by the fact that 3-oxosteroids of the 5α- and 5β-series (XXVI, XXVII) show Cotton effects of opposite signs; also that 7-oxosteroids of the 5α- and 5β-series show opposite Cotton effects, in spite of the fact that the configuration at a center α to the carbonyl group (C-8) is the same in both these latter compounds. See also the Octant Rule (Section VI).

4. α-Halogenoketones

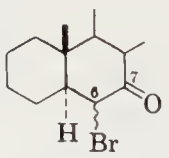
α-Halogen-substituted cyclohexanones show important conformational effects in their dispersion curves (10,14). Fluoro compounds, which behave differently from chloro and bromo compounds, and α-halogen-substituted cyclopentanones are considered on p. 290.

Equatorial α-chloro- or α-bromocyclohexanones give curves which are of the same sign as those of the parent ketones carrying no halogen substituent. For axial α-chloro- or α-bromocyclohexanones this is not necessarily true; here the stereochemistry of the group C:O·CX as a whole becomes dominant and controls the sign of the Cotton effect curve. This sign can be predicted by examining a model of the appropriate ketone in the following manner.

The cyclohexanone ring is placed in such a way that the carbonyl carbon atom is the "head" of the chair and is viewed along the O=C bond as shown by the arrow in LXXII. If the axial α-chlorine or



(LXXII)
α-Halo-
ketones



(LXXIII)
6-Bromo-7-
oxo-5α-
steroid

bromine atom lies to the *right* of the observer (LXXII, X = Cl or Br), the single Cotton effect will be *positive;* if the chlorine or bromine is to the *left* (LXXII, X′ = Cl or Br) the effect will be *negative.*

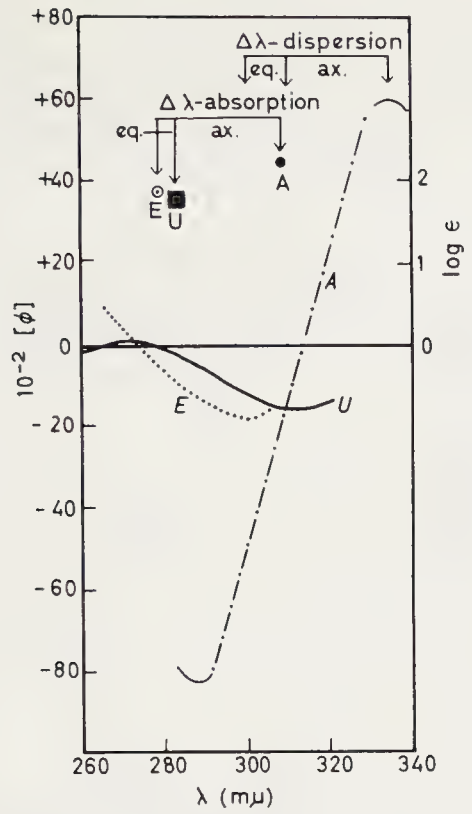


Fig. 6. α-Halogenoketones (14). Absorption and dispersion characteristics of a 7-oxo-5α-steroid and its 6-bromo derivatives (LXIII). The fragments of curves include the peaks and troughs of the dispersion curves. *U*, Unsubstituted 7-ketone; *E*, 6α(equatorial)-bromo-7-ketone; *A*, 6β(axial)-bromo-7-ketone. The separate points in the upper part of the figure show the absorption maxima.

Applications of this "axial α-haloketone dispersion rule" in problems of absolute configurations are discussed on pages 316–317.

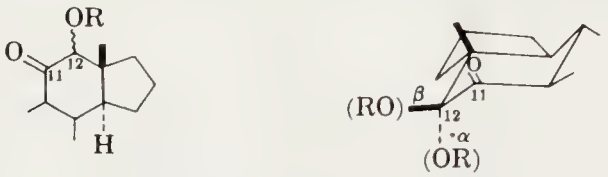
In addition to these gross changes in the form of dispersion curves caused by axial substituents, both axial and equatorial α-chlorine and α-bromine substituents cause changes in the wavelengths of the peaks and troughs of the Cotton effect curves (Fig. 6). Axial chlorine or bromine causes bathochromic shifts which can be correlated closely with the ultraviolet spectral changes of these groups previously studied by Cookson *et al.* (68,70). The corresponding equatorial substituents cause small wavelength shifts, usually hyposochromic.

Details, including comparisons with ultraviolet absorption maxima, are given in Table XI.

The signs of the Cotton effects for axial α-fluoroketones are found to be opposite to those for their chloro and bromo analogs. The only α-bromocyclopentanones so far studied are the 16α- and 16β-bromoandrostan-17-ones (14); here the terms "axial" and "equatorial" have no meaning. (See also 26,27.)

5. α-Ketols

A thorough survey carried out jointly by Djerassi, Tamm, Schindler, and their colleagues (15) has shown interesting conformational effects in the dispersion curves of α-ketols which parallel those found for



(LXXIV)
12-Hydroxy-(or acetoxy-)11-oxosteroid

TABLE XI
α-Halogenoketones
A. Wavelength Changes

Keto group	λ_{max} unsubstituted		$\Delta\lambda$ (substituted − unsubstituted)					
			Equatorial α-halogen[c]			Axial α-halogen[d]		
	D[a]	A[b]	Substituent	D[a]	A[b]	Substituent	D[a]	A[b]
3(5α)	307	286	2α-Cl	+ 3	−7	2α,2β-Cl_2	+18	+15
3(5α)			2α-Br	+ 4	−4	2α,2β-Br_2	+23	+12
3(5α)			4α-Br	− 4				
			2α,4α-Br_2	− 1				
			2α-Cl, 4α-Br	− 7				
6(5α)	306	280				5α, 7β-Br_2	+24	+25
6(5α)						5α,7α-Br_2	+56	+60
7[e](5α)	310	283	6α-Br	−10	−5	6β-Br	+25	+26
7(5α)						8β-Br	+25	
11	322	294				9α-F	+18	
11	325[f]					9α-Br	+25	
11						12α-F	+15	+17[f]
11						12α-Cl	+15	+17[f]
11						12α-Br	+20	+23
12	305	280	11α-Br	−10	−6	11β-Br	+32	

TABLE XI (*continued*)
B. Amplitudes, $10^{-2}a$

Keto group	Unsubstituted	Equatorial α-halogen[c]		Axial α-halogen[d]	
2	+77[f]			3α-Br	+272
3 (5α)	+65, +46	2α-Cl	+54	2α,2β-Cl_2	+147
3 (5α)		2α-Br	+62, +52	2α,2β-Br_2	+186
3 (5α)		4α-Br	+33		
3 (5α)		2α,4α-Br_2	+53, +34		
3 (5α)		2α-Cl,4α-Br	+41		
6 (5αH)	−76			5α,7β-Br_2	−282
6 (5αH)				5α,7α-Br_2	−178
6 (5αOH)	−110			7α-Br	+ 95
6 (5αOAc)	−88			7α-Br	+171
7	−15	6α-Br	−27	6β-Br	+143
7				8β-Br	−309
11	+12, +15[f], +52			9α-F	− 44
11				9α-Br	+253
11				12α-F	+117
11				12α-Cl	−116
11				12α-Br	−148, −182
12	+10	11α-Br	+15	11β-Br	−188

[a] Dispersion in methanol except as noted (14).

[b] Absorption in ethanol or ethanol–chloroform (80:20) (68).

[c] Includes diequatorial haloketones of type CX·CO·CX.

[d] Includes *gem*-dihaloketones (CX_2·CO), in which one halogen must be axial; also axial-equatorial and diaxial compounds of type CX·CO·CX.

[e] Formula LXXIII.

[f] In dioxane.

[g] Values for signs and amplitudes ($10^{-2}a$) of waves.

the ultraviolet absorption maxima (Cookson and Dandegaonker, 70; Baumgartner and Tamm, 50). The results for 11,12-ketols (LXXIV) and their acetates of the 14α-series are set out in Table XII. The wavelength changes, Δλ, show bathochromic and hypsochromic effects for axial and equatorial hydroxyl (or acetoxyl), respectively; these changes are closely similar for absorption maxima and for the peak of the dispersion curve (Fig. 7). So far as the amplitudes of the dispersion curves are concerned, the striking feature is the considerable increase in amplitude for axial α-acetoxy ketones. (These also show a

TABLE XII
11,12-Ketols (14α-Series)[a]

Curve[a]	Solvent[b]	λ_{max} unsubstituted	$\Delta\lambda$ (substituted − unsubstituted)			
			*e*OH	*e*OAc	*a*OH	*a*OAc
A. Wavelength Changes						
11-Ketones (cf. LXXIV) Dispersion	D	325	−10	−2	+12	+15
	M	320	−5	−8		+15
Absorption	A	298	−12	−5	+15	+10
12-Ketones Dispersion	D	312		−2	+20	+22
	M	305	−5		+22	
Absorption	A	290	−12	−5	+17	+8
B. Amplitudes of Dispersion Curves and Extinction Coefficients						
11-Ketones Dispersion, $10^{-2}a$	D	43	*44*[c]	*42*[c]	42	88
	M	12	14	28		73
Absorption, log ϵ_{max}	A	1.47	1.61	1.48	1.69	1.84
12-Ketones Dispersion, $10^{-2}a$	D	*30*[c]		*41*[c]	77	162
	M	10	*17*[c]		39	
Absorption, log ϵ_{max}	A	1.69	1.90	1.86	1.84	2.06

[a] Dispersion (ref. 15), absorption (refs. 50, 70).

[b] A = ethanol; D = dioxane; M = methanol.

[c] Values in italics are taken from dispersion curves where the trough was not reached; i.e., the true values may be *greater* than these.

considerable increase in log ϵ_{max}, although until further examples are available this should perhaps be looked on as a coincidence.)

Other ketols discussed (15) include the following steroid types (position of ketone group, first; hydroxyl group, second) 4,5; 6,5; 16,17; 17,16; 17,17*a* (*D*-homo); 17*a*,17 (*D*-homo); 20,17 (side chain); 20,21 (side chain); 20,17 (and 21) (side chain).

6. Solvent Differences

Comparison of molecular rotation values $[M]_D$ of two or more compounds in different solvents is always unsatisfactory because of the often unknown magnitude of solvent effects. Comparison of dispersion curves in different solvents is still more undesirable since solvents may cause differences in (*a*) the magnitude of the rotation at any given wavelength, (*b*) the wavelengths of peaks and troughs, (*c*) the amplitude of a single wave, and (*d*) the fine structure of multi-

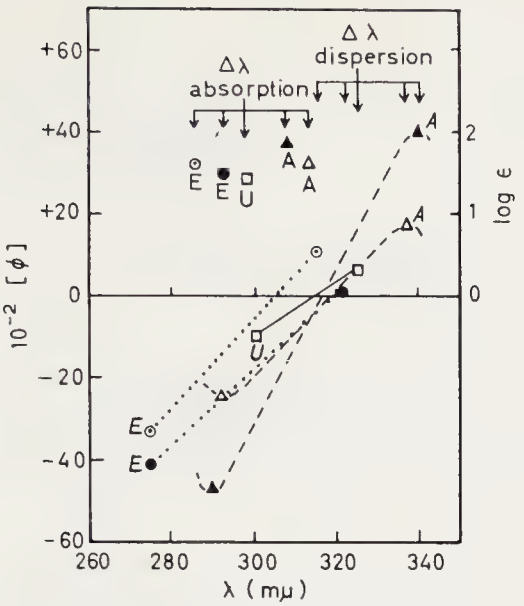


Fig. 7. α-Ketols (15). Absorption and dispersion characteristics of an 11-oxosteroid and its 12-hydroxy and 12-acetoxy derivatives (LXXIV). The fragments of the curves include the peaks and troughs of the dispersion curves. *U*, Unsubstituted 11-ketone; *E*, 12β(equatorial)-hydroxy- (○) and acetoxy- (●) 11-ketones; *A*, 12α (axial)-hydroxy- (△) and acetoxy- (▲) 11-ketones. The separate points in the upper part of the figure show the absorption maxima.

ple waves. The factor (*b*) is illustrated by the comparisons in Table VII, part A.

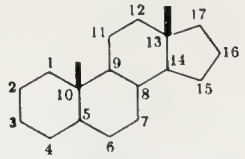
D. α,β-UNSATURATED KETONES

The multiple Cotton effect curves characteristic of α,β-unsaturated ketones (8,13) are at first sight much less amenable to systematic treatment than the simple curves given by saturated ketones. For the latter, the "waves" are clearly at or about the absorption maximum of the keto group at about 290 mμ; "peak" and "trough" wavelengths vary with λ_{max} (absorption) as expected (e.g., for α-haloketones, see Section III-C-4), and regularities in amplitude in different classes of ketones can readily be recognized (see Table VII).

The multiple Cotton effects of the α,β-unsaturated ketones at about 350 mμ are clearly related to the low intensity absorption maxima in the same region studied by Cookson (71). One or more extrema (peaks or troughs) always occur at the beginning of the absorption region (about 350–370 mμ). (The values discussed in this section

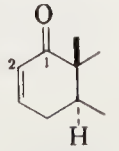
TABLE XIII

α,β-Unsaturated Ketones[a,b]

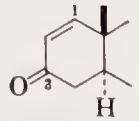


(LXXV)
Steroid numbering

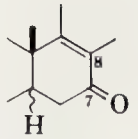
Compound	[ϕ] and λ		Sign and amplitude of wave, $10^{-2}a$	Ref.[d]
	Peak[c]	Trough[c]		
Cyclopentenones (and related systems)				
5α-Cholest-14-en-16-one, 3β-benzoyloxy-	+ 6,520 (360)	− 4,140 (305)	+107	13.XIV
5β-Pregn-16-en-20-one, 3α-acetoxy- (LXXX)	+ 3,560 (362)	− 3,740 (304)	+ 73	8.XLI
16-Methylene-androst-5-en-17-one, 3β-hydroxy- (LXXXVI)	+ 1,630 (394)	− *5,310* (310)	+ 69	8.LXV
5α-Guai-1-en-3-one derivative (tenulin) (LXXXVII)	− 1,650 (375)	+ 1,770 (312)	− 34	13.IIa
5α-Guai-1-en-3-one derivative (helenalin)	− 3,580 (365)	+ 1,270 (320)	− 48	13.VI
1β-Guai-4-en-3-one derivative (geigerin) (LXXXVIII)	− 1,650 (345)	+ 480 (300)	− 21	13.XVIII
1β-Guai-4-en-3-one derivative (isophotosantonic lactone)	− 200 (370)			13.XX



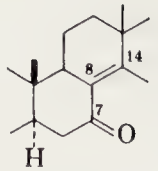
(LXXVI)
2-En-1-one



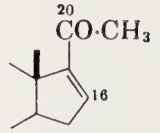
(LXXVII)
1-En-3-one



(LXXVIII)
8-En-7-one
(5α or 5β)



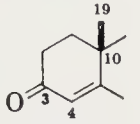
(LXXIX)
8(14)-En-7-one (5α)



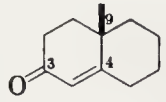
(LXXX)
16-En-20-one

Cyclohexenones

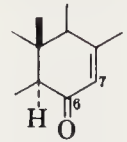
5α-Cholest-1-en-3-one (LXXVII)	− 810 (382)	+ 4,170 (312)	− 50	8.—
5α-Cholest-2-en-1-one(LXXVI)	− 1,520 (366)	+ 7,660 (311)	− 92	8.XXXVIII
Cholest-4-en-3-one(LXXXI)	− 630 (352)	+ *3,840* (325)	− *45*	3.XI
Androst-4-en-3-one, 17β-hydroxy-	− 810 (352)	+ *3,860* (325)	− *47*	8.XIII
19-Norandrost-4-en-3-one, 17β-hydroxy-	− 1,550 (351)	+ *4,880* (310)	− *64*	6.XXI



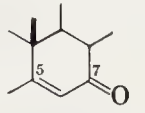
(LXXXI)
4-En-3-one



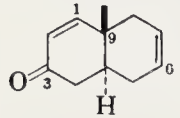
(LXXXII)
9-Methyl-Δ^4-octalone-3



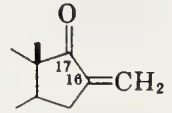
(LXXXIII)
7-En-6-one



(LXXXIV)
5-En-7-one



(LXXXV)
trans-9-Methyl-$\Delta^{1,6}$-hexalone-3

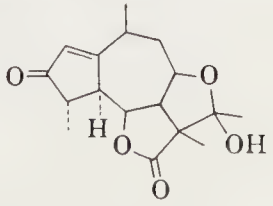


(LXXXVI)
16-Methyl-ene-17-one

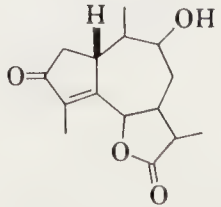
(*continued*)

TABLE XIII (*continued*)

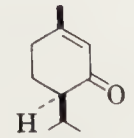
Compound	[ϕ] and λ		Sign and amplitude of wave, $10^{-2}a$	Ref.[d]
	Peak[a]	Trough[a]		
8α-Androst-4-en-3-one, 17β-hydroxy-	+ 3,420 (372)	− 1,910 (317)	+ 53	8.LV
Cholest-4-en-6-one	− 1,670 (359)	+ 6,180 (305)	− 78	7.LVII
Cholest-5-en-4-one	+ 890 (360)	− *6,100* (300)	+ *70*	7.LVI
Cholest-5-en-7-one, 3β-acetoxy- (LXXXIV)	+ 660 (357)	− *6,450!* (315)	+ *71!*	8.XXXII
5α-Ergost-7-en-6-one, 3β-acetoxy-Δ^{22}- (LXXXIII)	+ 5,600 (362)	− *9,100* (320)	+*147*	8.XXXIV
5α-Cholest-8-en-7-one, 3β-acetoxy- (LXXVIII)	+ 210 (369)	− *3,450!* (315)	+ *37!*	8.XXXVI
5β-Chol-8-en-7-one, 3α-acetoxy,24-oic (methyl ester) acid (LXXVIII)	− 850 (370)	+ 570 (332)	− 14	8.XXXV
5α-Cholest-8(14)-en-7-one, 3β-acetoxy- (LXXIX)	− 2,440 (375)	+ 700 (320)	− 31	8.XXXVII
5α-Spirost-8-en-11-one, 3β-hydroxy-	− 120 (375)	+10,100 (300)	−102	4.XXII
Cyclohex-2-en-1-one, 3-methyl-6-isopropyl- (piperitone) (LXXXIX)	− 740 (370)	+ 380 (329)	− 11	8.II
(−)-9-Methyl-3-oxo-*trans*-Δ-octalin	− 1,670 (380)	+ 2,300 (322)	− 40	E.16.V
(+)-9-Methyl-3-oxo-Δ^4-octalin (LXXXII)	+ 960 (362)	+ 3,400 (308)	− 24	E.16.VI



(LXXXVII)
Tenulin

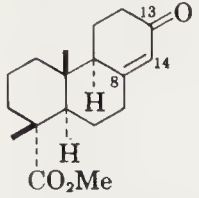


(LXXXVIII)
Geigerin

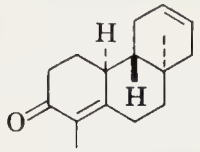


(LXXXIX)
Piperitone

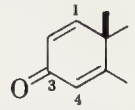
9-Methyl-4-oxo-$\Delta^{5(10)}$-octalin	+ 150 (362)	− 3,080 (312)	− 32	E.16.VIII
Eudesm-4-en-3-one (Δ^{11})	− 640 (354)	+ *3,880* (312)	− *45*	7.XXIV
7α-Eudesm-4-en-3-one (Δ^{11})	+ 2,640 (360)	− 320 (322)	+ 30	7.XXVa
Podocarp-8(14)-en-13-one derivative (XC)	− 2,290 (352)	+ *6,950* (312)	− *92*	7.XXII
Perhydrophenanthrenone derivative (XCI)	− 470 (351)	+ *2,460* (325)	− *29*	E.7.XXVII
Cross-Conjugated Dienones				
Androsta-1,4-dien-3-one, 17β-hydroxy- (XCII)	− 410 (366)	+ 910 (315)	− 13	8.XLIX
Cholesta-1,4-dien-3-one	− 260 (380)	+ 1,180 (322)	− 14	7.XXX



(XC)
Podocarpane derivative from abietic acid



XCI

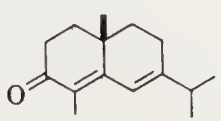
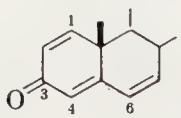
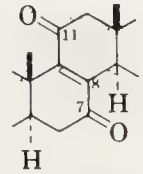


(XCII)
Steroid 1:4-dien-3-one

Extended Dienones				
Eudesma-4,6-dien-3-one (β-cyperone, XCIII)	+10,500 (384)	− 2,030 (337)	+125	7.XXXVII
Cholesta-4,6-dien-3-one	+49,900 (389)	*−12,500!* (315)	*+524!*	7.XXXIX
Cross-Conjugated Trienones				
Androsta-1,4,6-trien-3-one, 17β-acetoxy- (XCIV)	+ 5,100 (404)	−12,500 (325)	+173	8.LXVIII
Spirosta-1,4,6-trien-3-one	+ 3,950 (405)	−13,300 (335)	+172	4.XXVII

(*continued*)

TABLE XIII (*continued*)

Compound	[ϕ] and λ Peak[a]	[ϕ] and λ Trough[a]	Sign and amplitude of wave, $10^{-2}a$	Ref.[d]
	Enediones			
Cholest-4-ene-3,6-dione	− 2,380 (402)	+ 6,600 (307)	− 90	8.LXII
Spirost-8-ene-7,11-dione, 3β-acetoxy- (XCV)	− 180 (450)	+ 4,060 (330)	− 42	4.XXVIII
 (XCIII) β-Cyperone	 (XCIV) Steroid 1,4,6-trien-3-one	 (XCV) Steroid 8-ene-7,11-dione		

[a] In dioxane.

[b] *Inflections* and the amplitudes calculated from them are in *italics*. For curves where there is not even an inflection in the region of 310–320 mμ, the rotation at 315 mμ is chosen as an arbitrary value and is shown in *italics* followed by an exclamation mark (!).

[c] Values in parentheses are the wavelengths in mμ.

[d] Djerassi *et al.*; see Table IV.

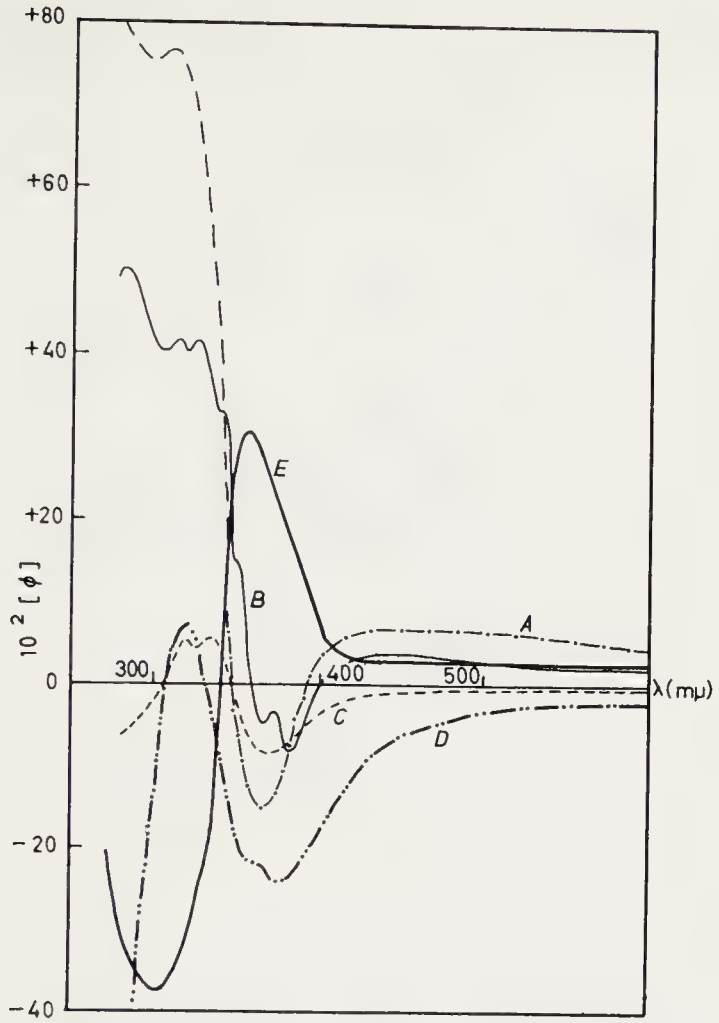


Fig. 8. α,β-Unsaturated ketones. Complete multiple waves (solvent: dioxane). *A*, 5α-Cholest-2-en-1-one (LXXVI); *B*, 5α-cholest-1-en-3-one (LXXVII); *C*, methyl 3α-acetoxy-7-oxo-5β-chol-8-enate (LXXVIII); *D*, 3β-acetoxy-5α-cholest-8(14)-en-7-one (LXXIX); also a single wave, *E*, 3β-acetoxy-5β-pregn-16-en-20-one (LXXX).

are in all cases for the *largest* peak or trough in this range.) The corresponding extrema at the lower end of the absorption region (about 300–320 mμ) are sometimes clearly defined, but often only a faint inflection in a steeply falling (or rising) curve occurs at this point (e.g., cholest-4-en-3-one). In a few exceptional cases, e.g., that of a steroid 16-en-20-one (LXXX), a single Cotton effect curve is found.

There is, in fact, every gradation in behavior between these two

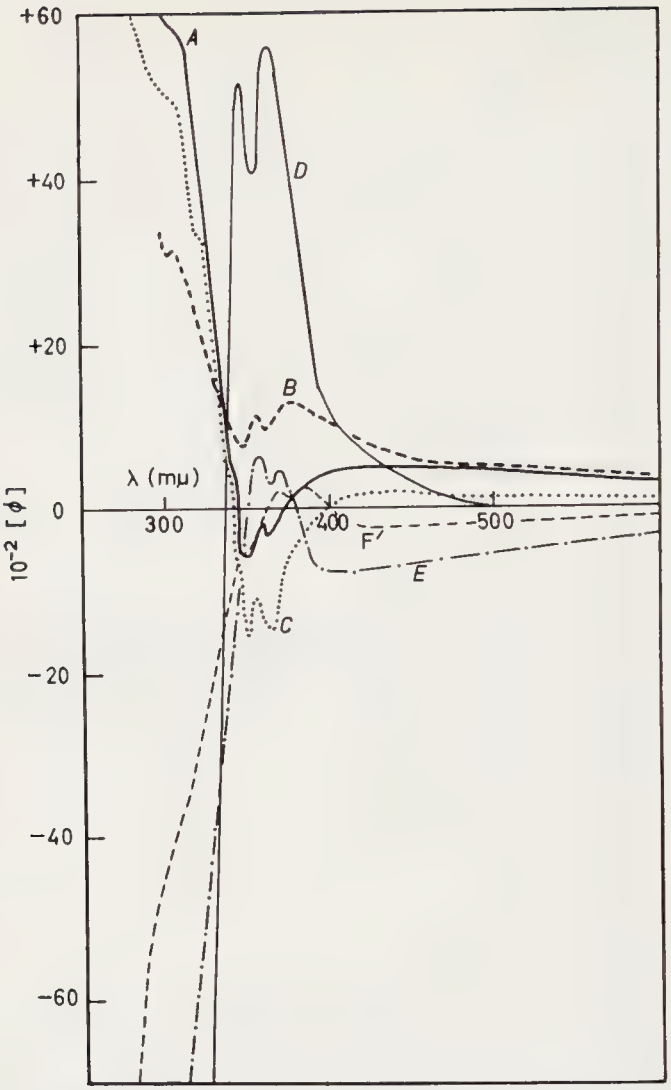


Fig. 9. α,β-Unsaturated ketones. Incomplete multiple waves (solvent: dioxane). *A*, Cholest-4-en-3-one (LXXXI); *B*, (+)9-methyl-Δ^4-octalone-3 (LXXXII); *C*, 17β-hydroxy-19-norandrost-4-en-3-one; *D*, 3β-acetoxy-5α-ergosta-7,22-dien-6-one (LXXXIII); *E*, 3β-acetoxycholest-5-en-7-one (LXXXIV); *F*, 3β-acetoxy-5α-cholest-8-en-7-one (LXXXVII).

extremes, and small changes in stereochemistry or in substituents may modify a curve so as to emphasize an inflection or, conversely, flatten out an extremum at ~320 mμ. These facts lead one to suggest that the extremum or inflection at about 320 mμ should be considered as significant, and that it should be used (together with the prominent

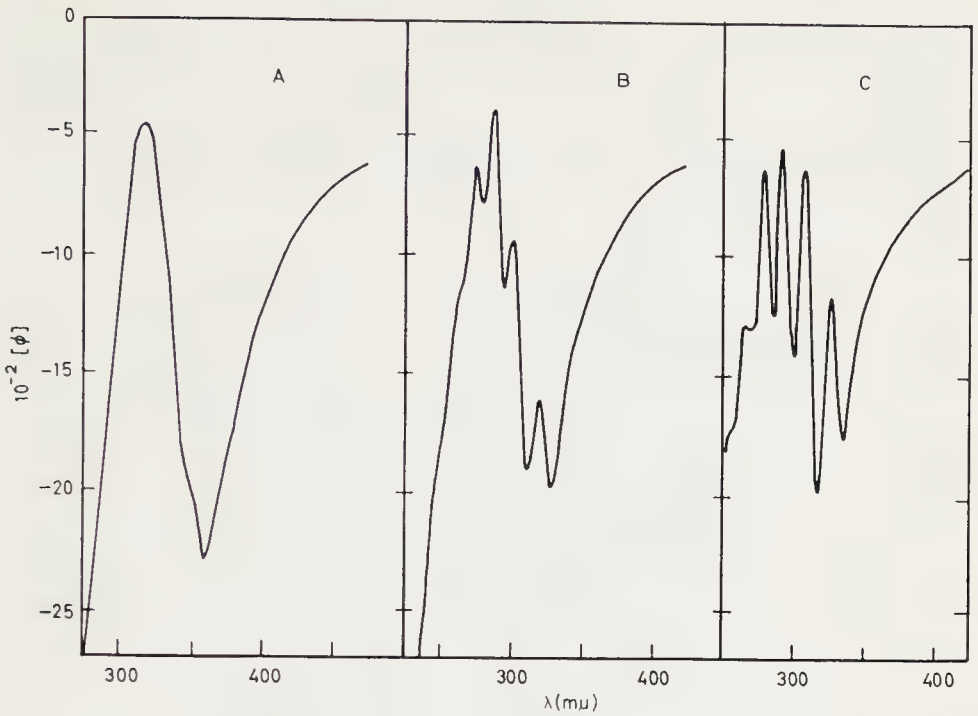


Fig. 10. α,β-Unsaturated ketones. (−)-*trans*-3-Oxo-9-methyl-$\Delta^{1,6}$-hexalin (LXXXV) in three solvents, showing development of fine structure with nonpolar solvents. *A*, In methanol; *B*, in dioxane; *C*, in octane. (Based on Fig. 1 of ref. 8.)

feature at about 360 mμ) to define the position, amplitude, and breadth of the α,β-unsaturated ketone wave.

(*a*) *Solvent Differences.* It may well be that, because of the complexity of the absorption spectra, no clear correlations of λ_{max} (absorption) and the midpoint of the dispersion wave will be possible. It is important to note that the absorption spectra (Cookson) are in hexane; dispersion curves (Djerassi) are usually in dioxane.

Cookson (71) pointed out that the low absorption maximum at ~350 mμ is shifted to longer wavelengths, and better resolved, when a hydrocarbon is used as solvent instead of an alcohol. Djerassi *et al.* (8) have found very striking differences between the dispersion curves of the same α,β-unsaturated ketone in methanol, dioxane, and octane. An example, (−)-*trans*-3-oxo-9-methyl-$\Delta^{1,6}$-hexalin (LXXXV), is illustrated in Figure 10. In methanol (the most polar of the three solvents) a smooth curve with one peak and one trough is obtained; in dioxane considerable fine structure appears; and in octane, still more.

For reasons of solubility, dioxane is to be preferred to octane as the standard solvent for α,β-unsaturated ketones.

Bathochromic shifts are found in the dispersion curves on going from polar to nonpolar solvents; e.g., for LXXXV trough at 361 (methanol), 362 (dioxane), 367 (octane); peak at 319 (methanol), 325 (dioxane), 330 mμ (octane).

(*b*) *Provisional Classification of Curves.* Data for representative curves are given in Table XIII. The following very tentative generalizations regarding relations between wave type and structure of the α,β-unsaturated ketone are the best that can be made at present. The terms "complete" and "incomplete" multiple wave are used provisionally for curves of the two types shown in Figures 8 and 9. ("Complete" implies that there is a definite extremum at ~320 mμ; "incomplete," that there is at most an inflection at this point.)

1. Cyclopentenones give complete multiple waves; the related semicyclic 16-en-20-one (LXXX) is unusual in giving a single wave.

2. Unsubstituted cyclohexenones (steroid 2-en-1-one and 1-en-3-one, LXXVI and LXXVII) give complete multiple waves.

3. 3-Substituted cyclohexenones in a terminal ring (e.g., the important steroid 4-en-3-one, LXXXI) generally give incomplete multiple waves.

4. The behavior of cyclohexenone groupings in the middle of a polycyclic system is irregular.

Little can be said as yet regarding the amplitudes of the waves; in general the 10^{-2} a values for the cyclohexenone types lie between 30 and 90.

A few values for compounds with three and four conjugated double bonds (dienones, etc., XCII–XCV) are given at the end of Table XIII.

IV. Applications of the Dispersion Method

A. RECOGNITION OF FUNCTIONAL GROUPS

The rotatory dispersion method can clearly be used as a means of recognizing the presence of carbonyl groups (even if chemically rather inert—e.g., in 11-oxosteroids). In most cases it will serve merely to confirm the results of infrared spectra, but there are a few special cases where the dispersion curve might be uniquely valuable. For example, the carbonyl groups of cyclopentanones and of acetoxyl groups absorb at nearly the same frequency (1749–1742 and 1742–1735

cm.$^{-1}$, respectively). Many compounds containing both functions show only *one* peak at about 1740 cm.$^{-1}$, e.g., 3-acetoxy-17-oxosteroids (Dobriner, Katzenellenbogen, and Jones, 89). In similar compounds containing an inert cyclopentanone carbonyl, the dispersion curve might be the only simple way of detecting the latter.

B. LOCATION OF FUNCTIONAL GROUPS (CARBONYL GROUPS)

In a series of compounds where adequate reference curves are available, the dispersion curve of a new compound may often give information which could not be obtained with any other physical tool. Thus, among steroid ketones of the cyclohexanone type, nearly every position has its characteristic type of "wave," as illustrated by Figures 4 and 5 and Table V. An example quoted by Djerassi and the Basel school (15) concerns a steroid monoketone (HPS 50) obtained by degradation of the cardiac aglycone tanghinigenin, the dispersion curve of which supports the hypothesis that the keto group (and therefore one point of attachment of the epoxide ring in the original aglycone) is at C-7. Further examples are rubijervine (12-oxosteroid, 179a), the "sodium-excreting factor" (20-oxosteroid, 170a) metagenin (7-oxo-5β-steroid, 191d) and bufotalinin (19-oxosteroid, 187b).

C. ALLOTMENT OF RELATIVE CONFIGURATION

The dispersion curves of polycyclic compounds (especially ketones) are largely dependent on stereochemistry; the curves can therefore often be made to allot the relative configuration of asymmetric centers.

An important example is the allotment of the β-configuration to the hydrogen atom at C-10 in 19-nor-Δ^4-3-oxosteroids, because their dispersion curves closely resemble those of the ordinary Δ^4-3-oxosteroids (8) (cf. Fig. 9).

A general case where the dispersion curve is of considerable value is in distinguishing between 3-ketones of the 5α- and 5β-series, e.g., certain reduction products of 19-nor-Δ^4-3-oxosteroids (17).

Since the principles involved here are essentially the same as those used for absolute configurations, further examples are given below (Section IV-D-3(b); Tables XV and XVI).

When more data have been collected, the amplitudes of ketone "waves" may serve to indicate whether a ketone group in a terminal ring is α or β to the ring junction (see Table VII, part B).

In all the above, the dispersion method may be considered as an extended and greatly improved version of the method of molecular rotation differences (Barton), since the rotation differences are being considered at hundreds of wavelengths instead of one.

D. PROBLEMS OF ABSOLUTE CONFIGURATION

1. General

Extensive use has been made in the past of molecular rotation differences (usually $\Delta[M]_D$ values) in the assignment of absolute configurations (130,132,163). In the future, comparison of rotatory dispersion curves will provide a much more sensitive tool for this purpose. Even with plain curves, the dispersion method has the advantage of multiplying the comparisons a hundred-fold, and the general trend of the curves is significant. When Cotton effects occur, the characteristic shapes of the curves make the method far more sensitive still. Vicinal action and conformational effects will certainly require more careful attention than in monochromatic studies (see Section IV-G).

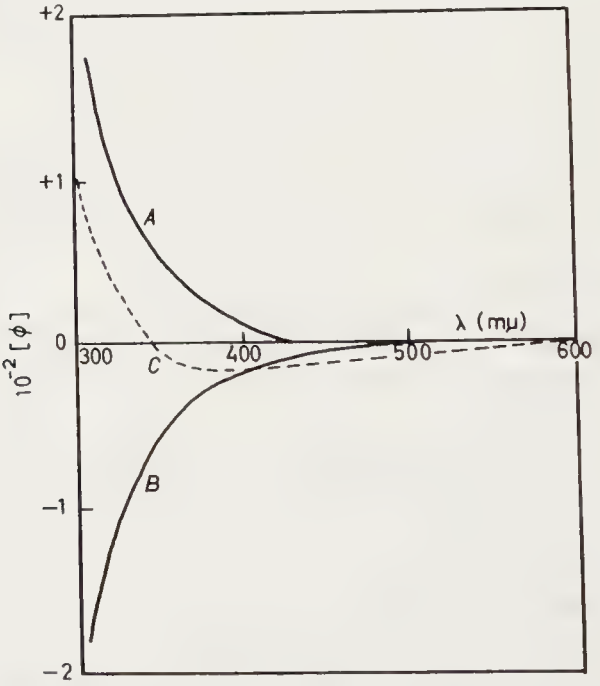


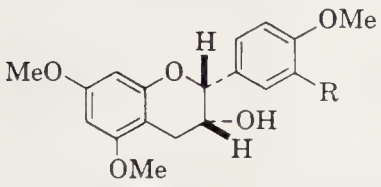
Fig. 11. Dibenzylcarbinols. *A*, XCVII (R = OMe) from (−)-epicatechin tetramethyl ether; *B*, EXCVII (R = OMe) from (+)-catechin tetramethyl ether; *C*, XCVII (R = H) from (−)-epiafzelchin trimethyl ether.

2. Plain Curves

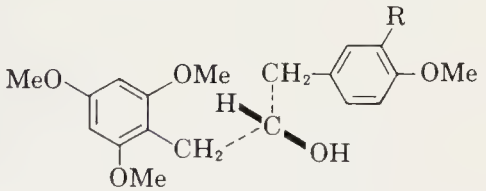
Three examples may be quoted:

1. The dispersion curves (Djerassi, 79) of certain degradation products of catechins (Birch *et al.*, 56) provide an excellent example of the use of plain dispersion curves for assignment of absolute configurations in a case where rotations at the sodium D line are inconclusive (Fig. 11).

The substituted dibenzylcarbinol (XCVII, R = OMe), which is obtained by ring opening of (−)-epicatechin tetramethyl ether (XCVI, R = OMe), has a plain positive dispersion curve. Its enantiomer, obtained from (+)-catechin tetramethyl ether, necessarily has an enantiomeric plain negative dispersion curve. Neither of these curves crosses the zero line. The carbinol (XCVII, R = H) obtained



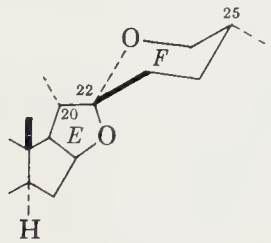
(XCVIa: R = OMe)
(−)-Epicatechin tetramethyl ether
(XCVIb: R = H)
(−)-Epiafzelchin trimethyl ether



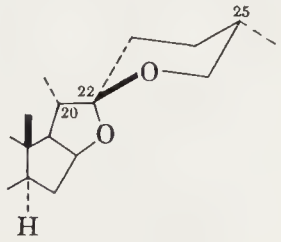
(XCVII)
Dibenzylcarbinol*

from (−)-epiafzelchin trimethyl ether (XCVI, R = H) has a negative $[M]_D$ value, but the general trend of its dispersion curve (which crosses the zero line at about 350 mμ) is positive, closely parallel to that of XCVII (R = OMe); (−)-epiafzelchin therefore has the *same* stereochemistry as (−)-epicatechin.

2. Although nearly all nonketonic sapogenins (XCVIII) have steeply falling negative curves (due to the spiroketal grouping; rings



(XCVIII)
22*a*-Spirostan

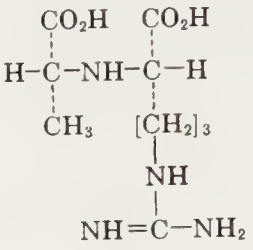


(XCIX)
22*b*-Spirostan

* For an important correction regarding the structure of XCVII, see J. W. Clark-Lewis, *Proc. Chem. Soc.* (*London*), *1959*, 388.

E and *F*) (4), one subgroup, the 22*b* sapogenins—e.g., cyclo-ψ- or *ana*-sarsasapogenin (XCIX)—have steeply rising *positive* curves. This is presumably a reflection of inverted stereochemistry at C-22, and is much more impressive than the $[M]_D$ value alone (see the discussions of the spiroketal system by Wall, Callow, and others, 65,195).

3. Greenstein and his group (113) have recently calculated the dispersion curves of individual centers in the isomeric octopines by the method shown in Section III-B, and have shown that the natural isomer is formed of D-alanine and L-arginine units (C).

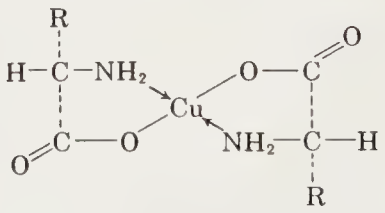


(C)
Octopine

4. A further example of the use of plain dispersion curves is in studies on emetine (194a).

3. Cotton Effect Curves

(*a*) *Copper Derivatives of Amino Acids.* One excellent example of configurational assignment from Cotton effect curves is found in the classical work of Pfeiffer and Christeleit (181,182) on the anomalous rotatory dispersion of the blue amino acid–copper complexes which absorb in the visible region of the spectrum; these authors showed that three groups of "natural" amino acids, which were not chemically correlated at that time, gave dispersion curves of the same general



(CI)
Copper complex of L-amino acid

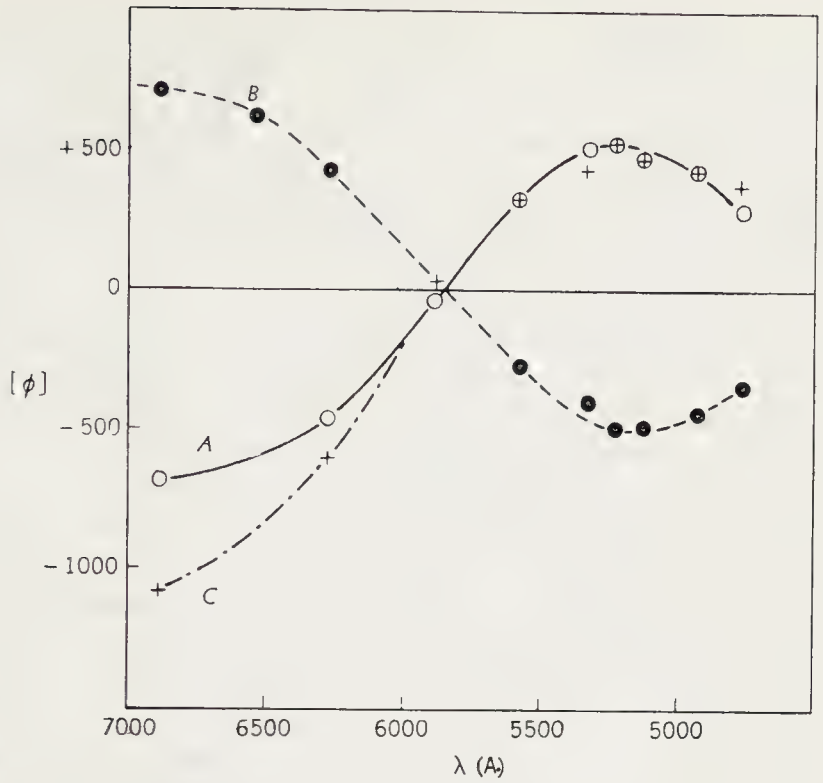


Fig. 12. Copper complexes of α-amino acids. *A*, L(+)-Valine; *B*, D(−)-valine; *C*, L(−)-phenylalanine. Curve *C* is almost identical with curve *A* from 600 to 500 mμ. (From Pfeiffer and Christeleit, 181, 182.)

pattern and concluded that all three groups had the *same* configuration at the α-carbon atom. Their curves for the complexes of L(+)- and D(−)-valine and of (−)-phenylalanine are reproduced in Figure 12. The general similarity of the (−)-phenylalanine curve to that of L(+)-valine indicates that these two acids have the same configuration. This provides an excellent illustration of the use of rotatory dispersion measurements in multiplying the analogies for a comparison; the curves of Figure 12 are more convincing evidence than two or three comparisons at scattered wavelengths.

The peaks for all the copper derivatives of the two groups of α-amino acids proper were at 522 mμ,the troughs were at or above 689 mμ (the longest wavelength studied); amplitudes were of the order of 10–20 $10^{-2}a$. The copper derivative of L(−)-proline, in which the nitrogen atom is a member of a ring and secondary in character,

shows a marked hypsochromic effect (peak, 484 mμ; trough, 572 mμ; $10^{-2}a$, 5).

Pfeiffer and Christeleit (182) (and simultaneously Karrer, 119), applied the copper complex method to show that (−)-glucosaminic acid has the D_s-configuration at C-2; the German authors further extended the method to α-hydroxy acids and found that copper salts of D-gluconic, D-galactonic, and D-lactic acids gave curves generally similar to that for copper D-valine.

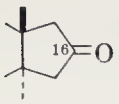
The Bethesda group (114) has recently shown that glutamic and aspartic acids and their amides follow the general pattern discovered by Pfeiffer and Christeleit. If the contributions of the α-centers in the copper complexes of diasymmetric amino acids (threonine, proline, etc.) are calculated, they also follow the same rules.

(*b*) *Ketones.* The results of the thorough survey of steroid ketones (Section III-C and Table V) led Djerassi to consider the use of their dispersion curves for solving problems of absolute configurations. A number of bicyclic ketones analogous to common steroid types and of known absolute configuration were examined (Table V) and each gave a single Cotton effect curve of the *same* sign as its steroid analogs. (Except for 9-methyl-*trans*-1-decalone; the steroid analogs of this behave abnormally.) This provided a sound basis for the generalization that "the typical features of dispersion curves of alicyclic monoketones are on the whole only a reflection of the immediate structural and stereochemical environment around that particular carbonyl group." This led to the conclusion that the generalized method of molecular rotation differences (Klyne, 126,127,133) could be extended to rotatory dispersion curves. The principles were expressed by Djerassi (7) as follows:

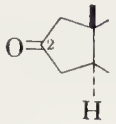
> (a) Terminal ring units of the same type make contributions to the rotatory dispersion curve which are, very approximately, independent of the nature of the rest of the molecule.
>
> (b) Each terminal unit can exist in two enantiomeric types which have rotatory dispersion contributions of opposite sign.

An excellent illustration of the second principle is provided by 16-oxo- and *A*-nor-2-oxosteroids (CII, CIII) the dispersion curves of which (see Fig. 5) are almost exactly mirror images.

One further general rule may be mentioned here—viz., that the substitution of methyl for hydrogen at a ring junction does not alter the *sign* of a Cotton effect curve. (This does not always apply to



(CII)
16-Oxosteroid

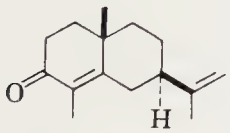
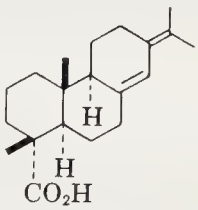


(CIII)
2-Oxo-*A*-nor
(5α)steroid

compounds where the carbonyl is α to the position affected by substitution.) It is also necessary to add that changes in conformation of the ring system may completely alter the sign of the Cotton effect (see discussion in Section IV-G).

Most of the correlations between the steroids on the one hand, and tri-, di-, and sesquiterpenes on the other, which were originally made by Klyne on the basis of monochromatic rotation differences, have subsequently been confirmed by rigid chemical proof (for references see 132,163). Compounds thus correlated can serve as a further support for the method of dispersion-correlations. Examples taken from the work of Djerassi (7) in which the dispersion curves take the expected form, are as shown in Table XIV.

TABLE XIV

Terpene	Steroid analog	Ref.[a]
α-Cyperone, Carrissone (CIV) (eudesmane series of sesquiterpenes)	Cholest-4-en-3-one	7.XXIV, 7.XXIII, 7.XXVI
 (CIV) α-Cyperone	 (CV) Neoabietic acid	
Tetrahydrosantonins 11β(H) analogs, tetrahydroartemisin	5α-(and 5β-)Cholestan-3-ones	7.XXXI, 7.XXXIII (cf. 63,134,67a,191c)
Friedelin	17a,β-Methyl-*D*-homoandrostan-17-one	7.XXXVI, 7.XXXV
Neoabietic acid (CV), unsat. ketone from	19-Nortestosterone	7.XXII, 7.XXI

[a] Djerassi *et al.;* see Table IV.

Other examples, in which the configurations have been allotted by the dispersion method, are as shown in Table XV.

TABLE XV

Terpene	Analog	Ref.[a]
ψ-Santonin 7-Hydroxy-1-oxosantanic acid	9-Methyl-*trans*-decalone-1	7.LXVII (cf. 66) 7.LXIX
Yohimbone	3-Oxo-19-nor-5α-steroid	7.LII,[b] 17.LIII
Trisnorhopanone	3-Oxo-*A*-nor-5β-steroids	90
3-Oxo-5β,10β-estrone derivatives	3-Oxo-5β-steroids	185a
19-Nor-14β,17α-steroid (4-en-3-one)	Steroid 4-en-3-one	83
Alantolactone derivatives	Methyl decalones and steroid 4-en-3-ones	53a,194b
Cadinol	Decalones	111a
4-Methyl-6-oxo-heptanoic acid (from actidione)	4-Methylhexan-2-one	24,92
Branched chain aldehydes (from phytol)	Branched chain aldehydes	29
Petasol (sesquiterpene)	$\Delta^{4(10)}$-Octalone-3	28
Gibberellic acid	2- and 3-Oxosteroids and methylhexahydroindanones	76a,191b
Hexahydroindanones and decalones	Methylated analogs	19,67b,183a
	Relative Configurations	
2-Oxo-19-norsteroids	2-Oxosteroids	92c
5-Methyl-3-oxo-19-norsteroids	3-Oxosteroids	31b
10-Hydroxy-3-oxo-19-norsteroids	"Ordinary" steroids (4-en-3-ones)	179b
Kogagenin	5β-Hydroxy-3-oxosteroid	185b,191d
18-Nor-17-oxosteroids	"Ordinary" 17-oxosteroids	152a
18-Nor-*D*-homo-17-oxosteroids	3-Oxosteroids	191a
Degradation product of lumisterol	3-Oxo-5β-steroid	65a
B-Nor-3-oxosteroids	"Ordinary" 3-oxosteroids	92b
Seredone	Alloyohimbone	182a

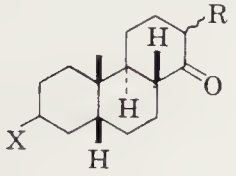
[a] Compound refs. are Djerassi *et al.;* see Table IV.

[b] Agrees with allotment from $[M]_D$ values (58,128).

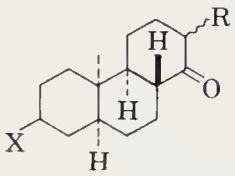
(*c*) *Some Intermediates in the Total Synthesis of Steroids.* In the course of the Oxford total synthesis of the steroids, Cornforth and Robinson (72) isolated and resolved certain *cis-syn-trans*-perhydrophenanthrene derivatives. The two enantiomeric series, called by

the original authors A(+) and A(−), must be represented by the formulas CVII, and CVIII, and the question arises which of the two formulas is to be allotted to which series. Several attempts were made by the present author to allot configurations to the two series by consideration of molecular rotation differences, but in no case were the results sufficiently definite to warrant publication.

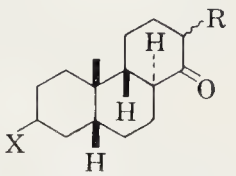
The problem was solved (9) by comparing the dispersion curves of compounds of the A(−) (unnatural) series with those of the B(+) (natural; *cis-anti-trans*) series (CVI) with respect to the optically active absorption bands of their keto groups.



(CVI)
B(+) (natural)



(CVII)
A(+)



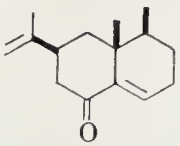
(CVIII)
A(−)

Steroid intermediates; des-*D*-14-ketones

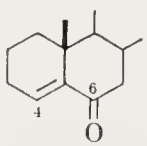
Two compounds of the A(+) series gave negative single Cotton effect curves, very similar to those given by members of the B(+), or natural, series. These curves permit the allotment with certainty of formulas CVII and CVIII, respectively, to the A(+) and A(−) series. This work illustrates how the rotatory dispersion technique gives a chear and definite stereochemical answer, while the older "monochromatic" method of rotation differences gives no answer at all.

(*d*) *Eremophilone.* The unsaturated sesquiterpene ketone eremophilone (now known to be CIX) is of particular interest in that it is one of the few smaller terpenes which does not follow the isoprene rule.

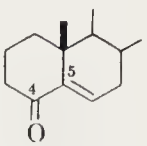
Djerassi *et al* (7) attempted to allot the absolute configuration of eremophilone by comparing its dispersion curve with those of steroid 4-en-6-ones and 5-en-4-ones (CX, CXI); they concluded that eremo-



(CIX)
Eremophilone



(CX)
Steroid 4-en-6-one

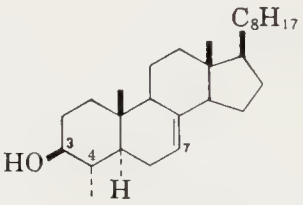


(CXI)
Steroid 5-en-4-one

philone was the *enantiomer* of CIX. Later chemical work from the same school (84,202) has shown that in fact, eremophilone is CIX; the failure of the dispersion method must be due to some conformational anomaly.

(*e*) *Lophenol.* An interesting example in which monochromatic rotation differences and dispersion curves complement one another is provided by the sterol lophenol, $C_{28}H_{48}O$ (Djerassi, Mills, and Villotti, 86). Reactions indicated a Δ^7-structure, but $[M]_D$ changes on modification of the 3-hydroxyl group were abnormal. Dispersion curves of related ketones showed that the stereochemistry of ring junctions was normal; therefore, some other structural change close to the 3-position was suspected.

Hindrance to hemiketal formation at C-3 (indicated by the fact that the dispersion curve of lophanone in methanol was unchanged on the addition of hydrochloric acid, see page 317) suggested substitution at C-2 or C-4, and direct comparison showed that lophanone was 4α-methyl-5α-cholestan-3-one. Lophenol is therefore 4α-methyl-5α-cholest-7-en-3β-ol (CXII).



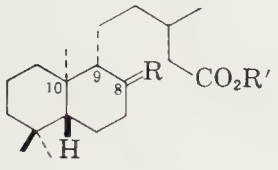
(CXII)
Lophenol

This investigation is notable for the number of new techniques used, including mass spectrometry and vapor phase chromatography in addition to those discussed above.

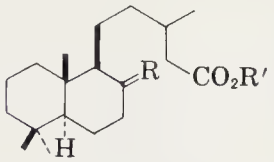
The biogenetic importance of a 4-monomethyl steroid (cf. cycloeucalenol, 76) as an intermediate between 4,4,14-trimethyl steroids of the lanosterol type and ordinary steroids is obvious (cf. Gautschi and Bloch, 99). Mazur, Weizmann, and Sondheimer (162) simultaneously announced the structure of citrostadienol, the 24-ethylidene derivative of lophenol.

(*f*) *Terpenes of Abnormal Stereochemistry: Diterpenes (eperuic and labdanolic acids).* The diterpene acid eperuic acid (King and Jones, 124) is the first higher terpene to be found which, while following the general structural pattern of di- and triterpenes and steroids, has the

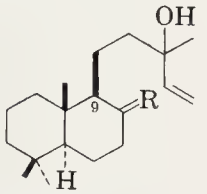
"abnormal" stereochemistry at C-10 (steroid numbering) shown in CXIIIa. The dispersion curves (12) of the very similar ketones obtained from eperuic acid on the one hand (CXIIIb), and from labdanolic acid (Cocker and Halsall, 67, CXIVb) and manool (CXVb) on the other hand, show clearly that eperuic acid has the "abnormal" configuration at C-10; CXIIIb shows a positive Cotton effect curve, while CXIVb and CXVb (the absolute stereochemistry of which is known) show negative curves.



(CXIIIa: R = CH_2; R' = H)
Eperuic acid
(CXIIIb: R = O; R' = Me)

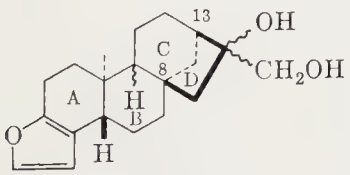


(CXIVa: R = Me, OH; R' = H)
Labdanolic acid
(CXIVb: R = O; R' = Me)

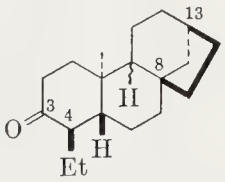


(CXVa: R = CH_2)
Manool
(CXVb; R = O; side chain at C-9 is $CH_2{\cdot}CH_2{\cdot}CO_2H$)

Cafestol. Parallel degradative studies by the schools of Djerassi (53) and of R. D. Haworth (109) indicated for cafestol a modified diterpene skeleton of the type CXVI (stereochemistry not implied) in which the *gem*-dimethyl group at C-4 had been changed into an ethyl group (subsequently cyclized to a furan). It was undecided whether the angle methyl group is at C-10 or C-5, until recently when Djerassi, Cais, and Mitscher (81) showed by several conformational arguments that the angular methyl group is in the usual C-10 position. The dispersion curve of the 3-ketone (CXVII) derived from cafestol



(CXVI)
Cafestol

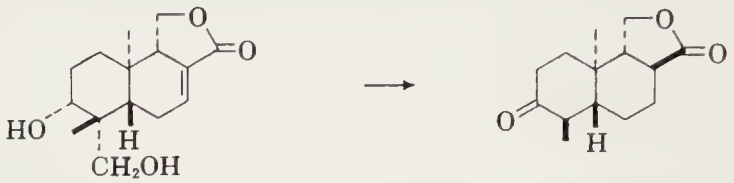


(CXVII)
Nor-3-ketone (from cafestol)

(CXVI) indicates, however, that it must be of a type *antipodal* to (+)-*trans*-3-decalone and 5α-cholestan-3-one or, to take a more precise analogy, 4α-ethyl-5α-cholestan-3-one. Cafestol is therefore the second diterpene shown to have stereochemistry antipodal to the usual

pattern. The configuration shown for the *C* and *D* rings is based on recent evidence.

Iresin. Djerassi and Burstein (80; cf. 82) have shown by arguments similar to those used for cafestol that the sesquiterpene iresin, which is an important link in the terpene biogenetic scheme, also has the "abnormal" stereochemistry (C-10 methyl group α) as shown in CXVIII.

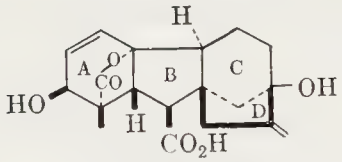


(CXVIII)
Iresin

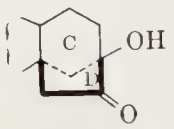
(CXIX)

The 13-norketone (CXIX) has a negative single Cotton effect curve, antipodal to that of 4α-methyl-5α-cholestan-3-one, and the curve of the related 2,6-dibromo-Δ^4-3-ketone is antipodal to that of the corresponding testosterone derivative (see also for relative configurations within the molecule the X-ray work of Rossman and Lipscomb, 185c).

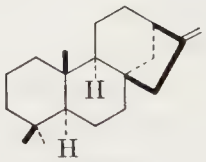
(*g*) *Gibberellic acid.* A series of rather involved stereochemical arguments by Grove, Stork, and their colleagues (76a,191b) have settled the configuration of the plant-growth substance gibberellic acid as CXX. The norketones of type CXXI obtained from this acid give positive Cotton effect curves; these provide us with a reference point for the important group of bicyclo-[3.2.1]-octan-6-ones. (See later arguments regarding phyllocladene, steviol, etc.)



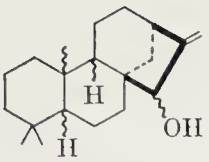
(CXX)
Gibberellic acid



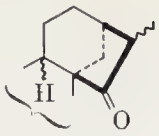
(CXXI)
Norketone from gibberellic acid



(CXXII)
Phyllocladene



(CXXIII)
Steviol



(CXXIV)
Isosteviol

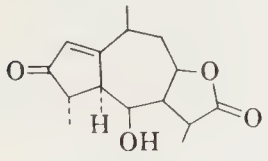
(*h*) *Phyllocladene.* The recent correlation of this diterpene with manoöl by Grant and Hodges (101a) shows that it has "normal" stereochemistry at the *A*/*B* ring junction. The positive Cotton effect curve given by the norketone (LXIX) resembles those of the similar bicyclo-[3.2.1]-octanones from gibberellic acid (CXXI). The two carbon bridge between C-8 and C-13 (the *D* ring) is thus β, and the complete formula of phyllocladene is CXXII; cf. Briggs *et al.* (62a) who produce additional evidence from ketones obtained by fission of ring *D*.

(*i*) *Steviol and the Garrya Alkaloids.* The diterpene steviol (CXXIII) can be converted into a ketone, isosteviol (CXXIV) which gives a negative Cotton effect curve (7). This indicates that the bicyclooctane position of the formula is as shown in CXXIII and CXXIV, enantiomeric type to CXXI; no definite conclusions can, however, be drawn about the *A* and *B* rings. Similar arguments may be applied to the *Garrya* alkaloids. The ketone cuachichicine (88, 197) also has a negative Cotton effect curve (7); its *C* and *D* rings must therefore be as in CXXIV.

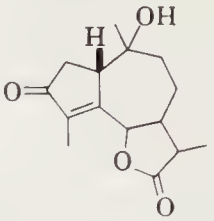
(*j*) *Sesquiterpenes* (*the Guaianolide Group*). Absolute configurations have recently been allotted tentatively by Djerassi, Osiecki, and Herz (13) to several members of the guaianolide group of sesquiterpenes on the basis of their dispersion curves.

Tenulin (LXXXVII) (46,62) and helanalin (CXXV) (64,111) are both cyclopentenones; their dispersion curves are of antipodal type to that of a steroid 14-en-16-one, and the absolute configurations at C-5 in the sesquiterpenes are therefore allotted as shown. Two reservations are necessary: (*a*) It has to be assumed that the methyl group at C-13 in the steroid and the hydrogen at C-5 in the guaianolides are equivalent. (This is known to be the case for cyclohexenones; see Table XIII and Fig. 9.) (*b*) It must be assumed that the rather heavily substituted cycloheptane ring *B* is equivalent to a cyclohexane ring. Both assumptions seem reasonable, although confirma-

tory evidence of configuration is desirable. Similar evidence indicates that geigerin (LXXXVIII) (180) and isophotosantonic lactone (CXXVI) (47) have the C-1β configuration.



(CXXV)
Helanalin



(CXXVI)
Isophotosantonic lactone

Rotatory dispersion evidence also indicates that a number of saturated ketones (dihydro derivatives) related to these unsaturated ketones are *trans*-fused β-hydrindanone-like structures of type CIII; all have positive single Cotton effect curves.

(*k*) *Possible Uses of the α-Haloketone Rule.* The finding (10,14) that in axially substituted α-chloro- and α-bromoketones the stereochemistry of the haloketone grouping determines the sign of the Cotton effect curve offers considerable opportunities in the determination of absolute configurations and other structural problems.

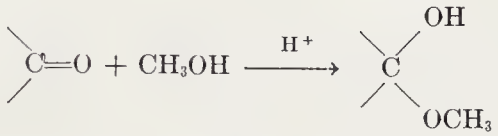
The determination of absolute configuration* for an axial α-haloketone comprises the following steps, all of which (except the first one, which may be unnecessary) can be carried out on a micro scale without using up any material: (*1*) if both α and α' positions can be substituted and the exact location of the halogen atom is unknown, then this must be established chemically; (*2*) the axial orientation of the α-halocyclohexanone must be confirmed by ultraviolet and infrared measurements; (*3*) the rotatory dispersion curve must be measured. It is then possible to determine in the manner illustrated above (cf. LXXII) which one of the two absolute configurations leads to the observed (positive or negative) Cotton effect.

Attention should be called to two further applications of rotatory dispersion measurements among α-halocyclohexanones. If the absolute configuration of the parent ketone is known but the location (α or α') of the axial halogen atom is not, then the rotatory dispersion curve will provide the answer. Conversely, if information concerning

* These two paragraphs are copied, almost verbatim, from *J. Am. Chem. Soc.*, *79*, 1506 (10).

the absolute configuration of the ketone and the location of the halogen substituent is available but spectroscopic determination of the orientation of the halogen atom is obscured by additional carbonyl groups, a decision may be reached by means of the rotatory dispersion curve. (See also 23,26,27.)

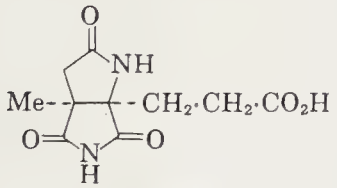
(*l*) *Hemiketal Formation.* A technique recently introduced by Djerassi, Mitscher, and Mitscher (22) depends on adding a small quantity of hydrochloric acid to a methanol solution of a saturated ketone and noting the change in the dispersion curve due to hemiketal formation.



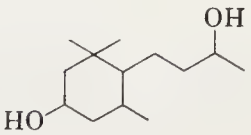
If hemiketal formation is complete, the ketonic "wave" in the dispersion curve disappears. If hemiketal formation is partial, the "wave" is diminished in amplitude; the extent and rate of diminution depend on several structural factors, such as ring size, and conformational factors (substitutions α and axial-β to the carbonyl group). See the example of lophenol (CXII) and page 312.

E. RECOGNITION OF OPTICAL ACTIVITY IN COMPOUNDS OF NEGLIGIBLE $[M]_D$ VALUE

An interesting side line of the work with the spectropolarimeter has been its use to "uncover" optical activity in some compounds which have negligible rotations at the D line but considerable activity at shorter wavelengths; examples are a degradation product of the vitamin B_{12} group (Clark *et al.*, 66a) (CXXVII) and two tetrahydroionanediols (Wright and Klyne, 200) from animal urines (CXXVIII).



(CXXVII)
Degradation product of vitamin B_{12}



(CXXVIII)
Tetrahydroionanediols

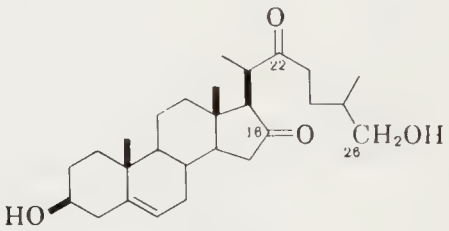
The bicyclic compound (CXXVII) has $[M]_D$ of $+1$, but $[M]_{365}$ is $-165°$. The diols (CXXVII) were first isolated from pregnant

mares' urine by Prelog *et al.* (183) who reported that they showed no measurable optical activity at the D line, although, as the Swiss workers stated, that might be due to internal compensation. Their diacetates and dibenzoates (the dispersion curves of which were kindly measured by Professor Djerassi) showed appreciable rotations over the range 500–300 mμ.

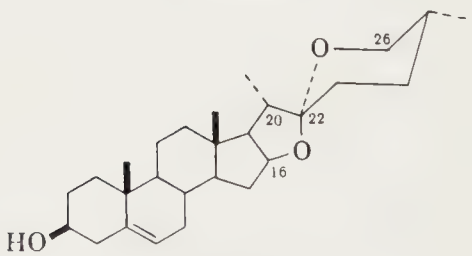
An earlier example deals with some apparently inactive chlorophyll derivatives (Pruckner, Oestreicher, and Fischer, 184).

F. ANALYTICAL USES

Some potential analytical uses are obvious for any property which is directly proportional to the concentration of a given substance X in solution. Since the molecular rotations of many compounds may be up to 100 times greater in the region of an absorption band then at the sodium D line, the gain in accuracy to be obtained by measuring the rotation at a "peak" or "trough" wavelength is very considerable. A good example quoted by Djerassi (11) is that of determining the amount of cryptogenin (CXXIX) as an impurity in diosgenin (CXXX) (an important starting material for large-scale preparation



(CXXIX)
Cryptogenin



(CXXX)
Diosgenin (spirost-5-en-3β-ol)

of steroid hormones). The differences in specific rotations for the D line is

$$[\alpha]_D(\text{Cryp.}) - [\alpha]_D(\text{Dios.}) = -70°$$

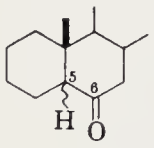
but at 320 mμ is

$$[\alpha]_{320}(\text{Cryp.}) - [\alpha]_{320}(\text{Dios.}) = -2600°$$

Calculation shows that contamination of diosgenin with 0.1% of cryptogenin would give a change in $[\alpha]_{320}$ of 0.026° (for a 1% solution of diosgenin in a 1 dm. tube).

Methods can doubtless be devised for the analysis of mixtures of more than two components by measuring the rotation at two or more selected wavelengths (cf. the extensive treatment of absorption spectra by Stearns in Mellon's *Analytical Absorption Spectroscopy*, 191; also 38).

Another analytical application of dispersion measurements suggested by Djerassi (11) is for the kinetic study of the isomerization of ketones. The 5α- and 5β-cholestan-6-ones (CXXXI) differ in $[\alpha]_D$ by only 40°, but for $[\alpha]_{310}$ the difference is over 1400°; the rate of isomerization of the unstable (5β) isomer by acid or alkali could therefore be followed very readily in the spectropolarimeter at 310 mμ.



(CXXXI)
6-Oxosteroids

The use of a photoelectric polarimeter (152) for the study of enzyme kinetics has been described by Levy and Cook (151).

Rotatory dispersion measurements have been used in kinetic studies on octahedral complexes by Brown and Ingold (62b).

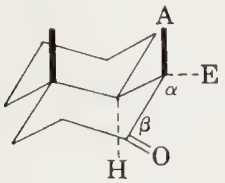
G. CONFORMATIONAL FACTORS

1. Saturated Ketones

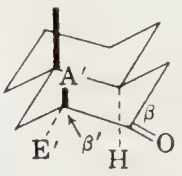
A long recent paper by Djerassi, Halpern, Halpern, and Riniker (17) covers many different conformational effects in saturated and unsaturated ketones by cyclohexanone type, chiefly those due to alkyl substituents and double bonds. Space here permits only a discussion of the principal effects.

Important additional work on triterpenes has been published recently (25); many of these conformational problems can now be explained in terms of the Octant Rule (Section VI).

(*a*) *Axial Methyl Effect.* The effects of introducing methyl groups in a large variety of positions and configurations relative to the keto groups of α- and β-decalones were studied. One of the most striking effects was obtained when an axial methyl group was introduced into a β-decalone at the α-position lying between the keto group and the ring junction (A in CXXXII). This caused a reduction in the amplitude of the ketone wave, whereas the introduction of the corresponding equatorial methyl (E) had little or no effect.



(CXXXII)
trans-β-Decalone
(substituted at α)



(CXXXIII)
trans-β-Decalone
(substituted at β')

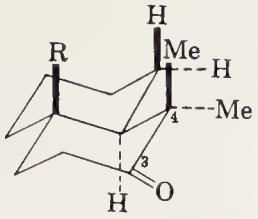
Introduction of methyl groups (equatorial or axial) at the other position adjacent to the β-keto group (A′ or E′ at β' in CXXXIII) had little or no effect. A *gem*-dimethyl group at this β' position caused some increase in the amplitude of the ketone wave. Examples are as follows (cf. 17).

Compound	$10^{-2}a$
5α-Cholestan-3-one	+45
4α-Methyl substituted (E in CXXXII)	+54!
4β-Methyl substituted (A in CXXXII)	+ 7
2α-Methyl substituted (E′ in CXXXIII)	+62
2β-Methyl substituted (A′ in CXXXIII)	+73
2α,2β-Dimethyl substituted (*gem*-dimethyl, A′ + E′ in CXXXIII)	+80

Other examples are found in the tetrahydrosantonins and tetrahydrosantonic acids* (31) and in the 17*a*-methyl-17-oxo-*D*-homosteroids (185).

(*b*) *4,4-Dimethyl Effect.* A much more striking effect on the dispersion curves of β-decalones is that shown by 4,4-dimethyl-*trans*-decal-3-ones of the lanostan-3-one type (CXXXIV).

* The dispersion curves have been used for allotment of configuration here (cf. p. 309).



(CXXXIV)
trans-β-Decalone
(lanostanone type
with 4,4-dimethyl)

"Ordinary" 5α-steroid-3-ones and their bicyclic analogs (CXXXII, R = Me), as also their 19-nor analogs (CXXXII, R = H), all show *positive* single Cotton effect curves, the amplitudes being affected by substitution at C-2 and C-4 as indicated in the preceding section.

The 4,4-dimethyl-5α-3-ones of type CXXXIV (whether they have the customary angular methyl group at C-10 or not) show *negative* single Cotton effect curves, unless the position is further complicated by double bonds or substituents in ring *B*.

Examples of $10^{-2}a$ values are as follows (cf. 17):

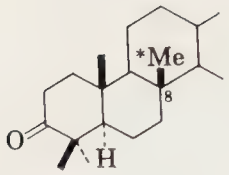
	4,4-H_2 series (CXXXII)	4,4-Me_2 series (CXXXIV,
5α-Steroid-3-one	+65, +45	−12,* −16**
19-Nor-5α-steroid-3-one	+90!	−20, −15
9-Methyl-*trans*-decalone	+47	−11

The reason for this reversal of sign is not clear, but it would appear to be due partly to a "2-alkylketone" effect (129) between C-4 and C-3 and partly to a "peri" effect between C-4 and C-6 (cf. CXXXIV).

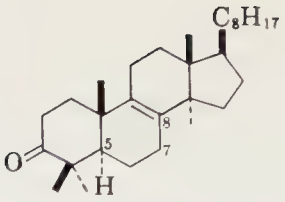
The complexity of the factors involved in determining the sign of the Cotton effect of a 3-keto group in the 4,4- dimethyl series is further illustrated by the following (cf. Table IX): (*1*) Introduction of an 8β-methyl group (starred in CXXXV), as in the common pentacyclic triterpenes, e.g., "α"- or "β"-amyrone) gives a *positive* curve. (*2*) Moving the double bond in the lanostane series from 7,8 (negative curve) to 8,9 also gives a *positive* curve (CXXXVI) (5α-cholest-7-en-3-one and -8-en-3-one both give positive curves). (*3*) Insertion of a

* Without 14α-methyl.

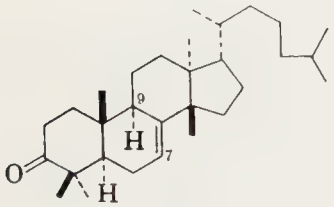
** With 14α-methyl (lanostane series).



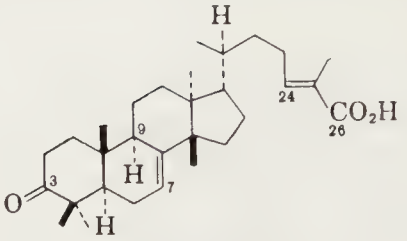
(CXXXV)
Amyrone type (with 8β-methyl)



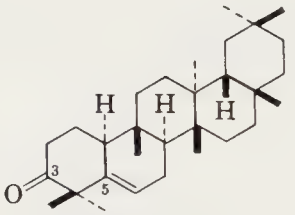
(CXXXVI)
Lanost-8-en-3-one



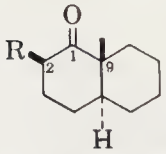
(CXXXVII)
Dihydrobutyrospermone



(CXXXVIII)
Masticadienonic acid (3-Oxotirucalla-7,24-dien-26-oic acid)



(CXXXIX)
Alnusenone



(CXL)
2,9-Dialkyl-*trans*-decalone-1

double bond at C-5 in the lanostane or 19-norlanostane series gives a positive curve.

The value of the dispersion method for stereochemical allotments in this series is illustrated by the following examples: (*1*) The close similarity between the curves for lanost-7-en-3-one and dihydrobutyrospermone (CXXXVII) (77,144) is strong evidence in favor of the 9α-H configuration of the latter. (*2*) The curves for masticadienonic acid (CXXXVIII) and its iso acid (49,188) are related in the same

way as those of lanost-7-(and -8-)en-3-one; this again indicates the 9α-configuration in the Δ^7-acid. (*3*) The antipodal relationship between the curves for alnusenone and 4,4-dimethyl-19-norcholest-5-en-3-one supports the 10α-H configuration (CXXXIX) allotted to alnusenone (75,190).

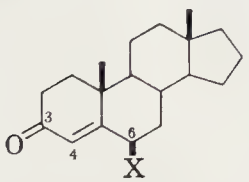
A start has been made in correlating the form of the Cotton effect curves for various 4,4-dimethyl-3-ketones with their rates of condensation with benzaldehyde which Barton *et al.* (45,48) have studied as a measure of conformational distortion.

Other interesting compounds studied include the 2,9-dialkyl-1-decalones (the diaxial isomer (CXL) of which shows an inversion of the Cotton effect) and the 30-nortaraxastan-20-ones (LXVI) (in which the dispersion curves follow the abnormal stability order; cf. 34).

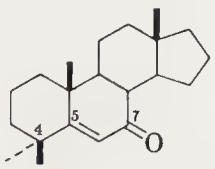
2. α,β-Unsaturated Ketones

Djerassi and his colleagues (17) have considered an extensive range of α,β-unsaturated ketones and have discovered a number of examples of *inversion* of the multiple Cotton effect which must be due to conformational (steric) effects of one kind or another.

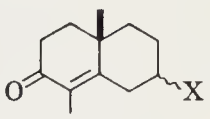
It is logical to consider first the unsaturated analogs of the axial α-methyl ketones—i.e., the axial γ-substituted α,β-unsaturated ketones of general type CXLI. Compounds of type CXLI (6β-substituted testosterones), with X = Cl, Br, or Me, show positive multiple Cotton effect curves—i.e., the usual negative curve characteristic of



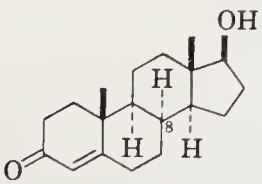
(CXLI)
6β(axial)-Substituted 4-en-3-one



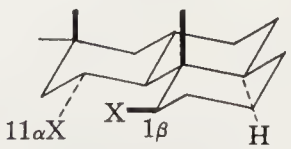
(CXLII)
4,4-Dimethyl-5-en-7-one



(CXLIII)
α-Cyperone and related compounds



(CXLIV)
8α-Testosterone



(CXLV)
Interference between 1β- and 11α-substituents

steroid 4-en-3-ones is inverted. The 6β-fluoro and 6β-hydroxy analogs (X = F, OH) give negative curves, as do the various 6α-(equatorial)substituted epimers of all types.

Compounds of enantiomeric type in which an axial γ-methyl causes inversion are the 4,4-dimethylcholest-5-en-7-one derivatives (CXLII) (*negative* curve; the compounds without *gem*-dimethyl have positive curves). The substitution of an axial methyl group in the α'-position, e.g., 2,2-dimethyltestosterone, does *not* invert the dispersion curve of a 4-en-3-one.

The next examples illustrate inversions caused by axial substitution at a greater distance; that is, by an isopropenyl or similar group at C-7, δ to the carbonyl at C-3. α-Cyperone (CXLIII, X = —CMe:$CH_2\beta$, equatorial) has the customary negative curve; its 7-epimer epi-α-cyperone (CXLIII, X = —CMe:$CH_2\alpha$, axial) (McQuillin, 161), has an inverted curve. The same behavior is observed with one of the 3-oxo-santon-4-enic acids of Abe (31a) (CXLIII, X = CHMe·$CO_2H(\alpha)$) where the 7-epi compound shows an inverted curve. A *methyl* group at C-7 is not, however, sufficient to invert the Cotton effect, although it modifies the shape of the curve.

Another striking example of the inversion of a multiple Cotton effect curve is provided by 8α-testosterone (CXLIV) in which one ring (presumably *B*) has become a boat. Djerassi (17) suggests that the other examples of inversion detailed above *may* perhaps be due to transformation of ring *B* to a distorted boat; he draws attention to the fact that all the inversions occur on modification of the adjacent ring to which the α,β-unsaturated ketone is exocyclic. Modification of the same ring (as in a 2,2-dimethyl-4-en-3-one) or of the adjacent ring when the conjugated group is not exocyclic to it (e.g., 4,4-dimethyl-8-en-7-one) has no major effect.

Other examples discussed include 1β-methyl and 11α-methyl steroids (interference between 1- and 11-positions has been found in other aspects of steroid chemistry) and 1,4-dien-3-ones of the santonin series.

3. Conformational Mobility

A recent study by Djerassi and Geller (18) provides a most striking example of the value of dispersion curves in the study of mobile conformations (cf. 33). The rotatory dispersion curve of 2-chloro-5-methylcyclohexanone was measured in several solvents; in octane

(nonpolar) the Cotton effect curve was negative, whereas in methanol (polar) it was positive. This may be ascribed to the fact that in the nonpolar solvent the compound exists predominantly in conformation CXLVIa and in a polar solvent predominantly in conformation CXLVIb. The infrared and ultraviolet spectroscopic results support these arguments (cf. 26,27).

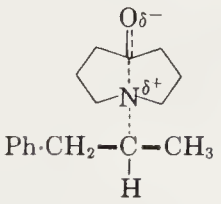
(CXLVIa; diaxial, in octane) ⇌ (CXLVIb; diequatorial, in methanol)

Conformations of *trans*-2-chloro-5-methylclohexanone

Djerassi *et al.* (30) have recently used the α-haloketone rule to demonstrate the presence of a boat form in 2α-homo-2β-methyl-5α-cholestan-3-one.

4. Transannular Interaction

An interesting example in which transannular interaction between nitrogen and carbonyl in an eight-membered ring causes abnormal rotatory dispersion has recently been discovered by Leonard, Djerassi, and their colleagues (20). The ketone CXLVII shows a negative Cotton effect superimposed on a positive plain curve; its open-chain analog shows a plain curve.



(CXLVII)

V. Algebraic Treatment of Curves

A. EFFECT OF "BACKGROUND"

The major features of Cotton effect curves are so pronounced that they can hardly be masked even by steeply rising or falling curves due to nonabsorbing groups in the molecule. For quantitative comparison of amplitudes, however, it is sometimes necessary to subtract

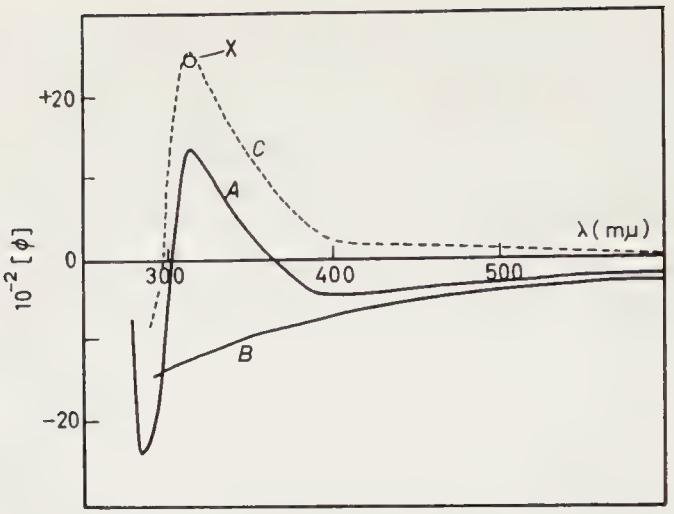
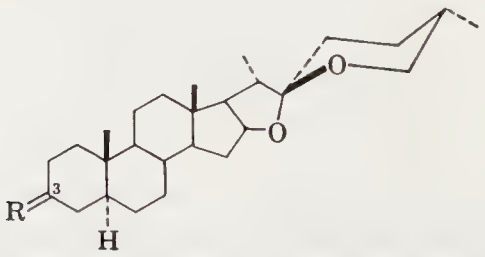


Fig. 13. Substraction of "background" curve (solvent: dioxane). *A*, 5α-Spirostan-3-one (CXLVIII; R = O); *B*, 5α-spirostan (CXLVIII; R = H_2); *C* (= *A* − *B*), contribution of 3-keto group. Point marked *X* is the peak of the curve for 5α-androstan-3-one, which is almost identical with curve *C*.

from the dispersion curve of a ketone (represented as *SA*) the curve for the corresponding deoxo compound or alcohol (represented as *S* = skeleton). The subtraction may be represented as an equation

$$RD_A = RD_{SA} - RD_S$$

This is shown in Figure 13 for 5α-spirostan-3-one and 5α-spirostan; the difference curve which represents the rotation contribution of the keto group (*A*) alone (in its asymmetric surroundings) is almost superimposable on that of 5α-androstan-3-one. The dispersion curve of 5α-androstane itself can barely be distinguished from the zero line.



(CXLVIIIa: R = O) 5α-Spirostan-3-one

(CXLVIIIb: R = H_2) 5α-Spirostan

B. VICINAL ACTION BETWEEN TWO ABSORBING FUNCTIONS

In studies of vicinal action between two functions in the same compound, the calculated dispersion curve of a bifunctional compound

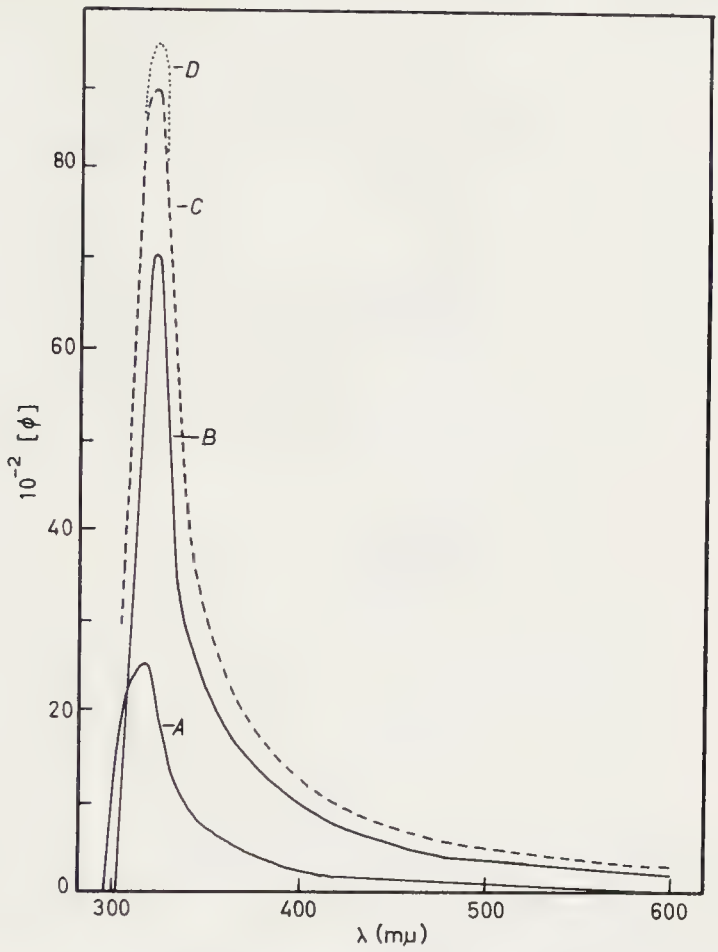


Fig. 14. Vicinal action (absence) (solvent: dioxane) (cf. 6). *A*, 5α-Androstan-3-one; *B*, 5α-androstan-17-one; *C*, 5α-androstane-3,17-dione (CXLIX) (experimental); *D*, "calculated" curve for 3,17-dione (identical with curve *C* except for small portion shown).

(*AB*) should be constructed as follows: If RD_A and RD_B are the rotations of the two appropriate monofunctional compounds, then

$$RD_{AB} = RD_A + RD_B$$

(*not* $^1/_1(RD_A + RD_B)$ as indicated previously) (6). If the skeleton

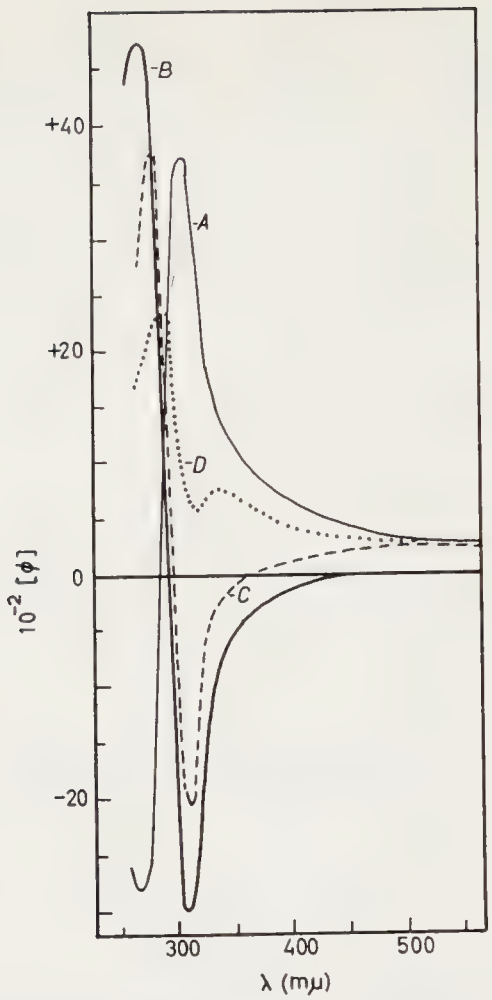


Fig. 15. Vicinal action (presence) (solvent: methanol) (cf. 6). *A*, 5α-Cholestan-3-one; *B*, 5α-cholestan-6-one; *C*, 5α-cholestane-3,6-dione (CL) (experimental); *D*, "calculated" curve for 3,6-dione.

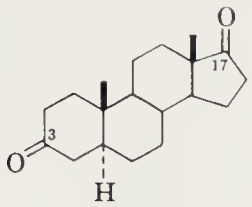
S has a significant contribution, RD_S, then the calculation should be done as follows

$$RD_{SAB} = RD_{SA} - RD_{SB} - RD_S$$

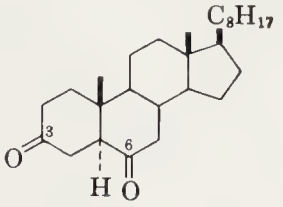
This is the same procedure as has been commonly used for monochromatic rotation differences (Barton).

Two examples are shown in Figures 14 and 15 (cf. 6). Figure 14 shows the calculated curve for 5α-androstane-3,17-dione, which is almost identical with the experimental curve; i.e., there is, as expected, no vicinal action between the two well-separated carbonyl groups.

Figure 15 shows similar curves for 5α-cholestane-3,6-dione; here the calculated curve is quite different from the experimental one, indicating serious vicinal action.



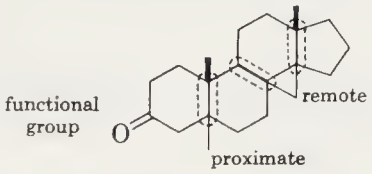
(CXLIX)
5α-Androstane-3,17-dione



(CL)
5α-Cholestane-3,6-dione

C. COMPARISON OF POLYCYCLIC KETONES AND BICYCLIC ANALOGS (See also the Octant Rule, Section VI)

An approach which may yield interesting results is a comparison of the dispersion curves given by polycyclic ketones and those of their



(CLI)
Proximate and remote centers of asymmetry

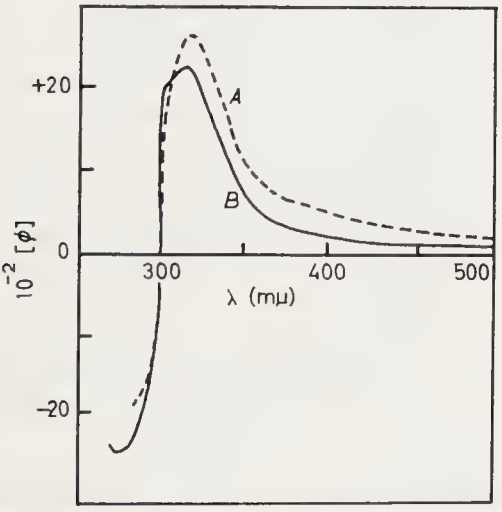


Fig. 16. Comparison of steroid ketones with bicyclic analogs (solvent: dioxane) (85,131). *A*, 5α-Cholestan-3-one (CLII); *B*, (+)-*trans*-9-methyldecalone-3 (CLIII).

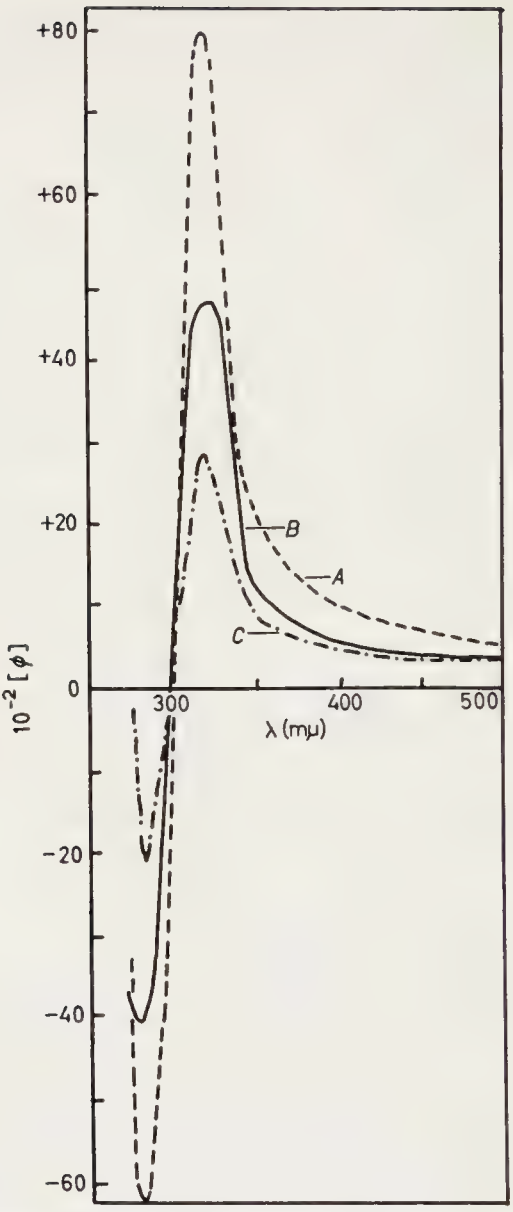
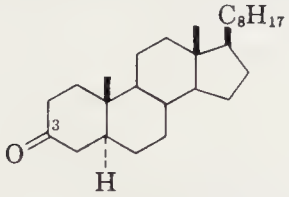


Fig. 17. Comparison of steroid ketones with bicyclic analogs (solvent: dioxane) (85,131). *A*, 5α-Androstan-17-one (CLIV); *B*, (+)-trans-8-methylhexahydroindan-1-one (CLV); *C*, difference curve (*A* − *B*).

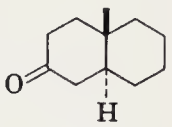
simple bicyclic analogs (131). Let us consider compounds in which the functional group is in a terminal ring, as in formula CLI. The ring junctions by which the ring carrying the functional group is joined to the rest of the molecule may be called the *proximate* centers

of asymmetry, and the others the *remote* centers. Comparison of bicyclic compounds and their polycyclic analogs shows that in some cases the dispersion curve for a polycyclic substance is essentially that due to the proximate centers of asymmetry. In other cases the remote centers have a profound effect, which is the cause of some of the "anomalies" found in previous work.

If dispersion curves of a 3-oxosteroid of the 5α-series (CLII) and its bicyclic analog, the methyl-*trans*-β-decalone (CLIII), are compared, it is found that they are almost superimposable (Fig. 16). We may tentatively interpret this as meaning that the fusion of rings *C* and *D* onto the bicyclic system makes no difference to the rotatory dispersion—or, in other words, that the rotatory dispersion of the 5α-3-oxosteroid is almost entirely due to interaction between the proximate centers C-5 and C-10 and the carbonyl group at C-3.

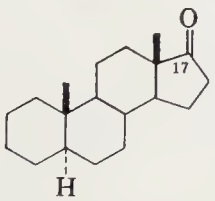


(CLII)
5α-Cholestan-3-one

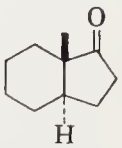


(CLII)
trans-9-Methyl-decalone-3

The relationship is quite different if we consider a 17-oxosteroid (CLIV) and its α-hexahydroindanone analog (CLV). Here the two curves (Fig. 17) differ very significantly in amplitude and the difference (Δ curve) may be plotted algebraically (see the work of Aulin-Erdtman (38) on absorption spectra). It is appreciated that the division of a rotation into components ascribed to individual centers *may* be of doubtful physical meaning because of vicinal action, but, if this proviso is constantly borne in mind, there seems no harm in sug-



(CLIV)
5α-Androstan-17-one



(CLV)
trans-8-Methyl-hexahydro-indan-1-one

gesting that the Δ curve represents *formally* the interaction between the remote centers C-8, C-9, etc., and the carbonyl group at C-17 in CLIV.

The last example (Fig. 18) introduces one of the ketosteroids which give curves of grossly unusual character, that is, the 17*a*-oxo-*D*-homosteroid (CLIV), which must be compared with the corresponding *trans*-α-decalone (CLVII). The dispersion curve for the 17*a*-ketone is abnormal, in that it does not show the characteristic ketone

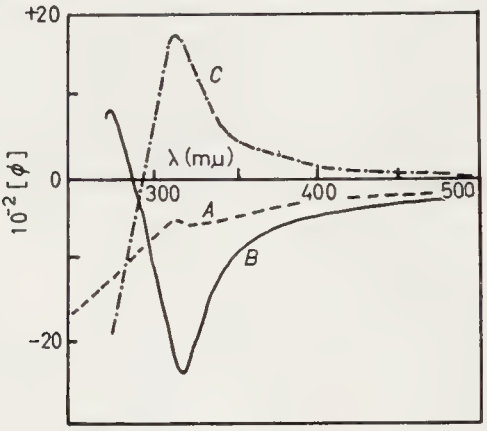
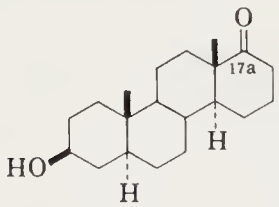
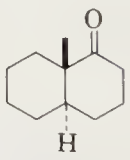


Fig. 18. Comparison of steroid ketones with bicyclic analogs (85,131). *A*, 3β-Hydroxy-5α-*D*-homoandrostan-17*a*-one (CLVI) in methanol; *B*, (−)-*trans*-9-methyldecalone-1 (CLVII) in dioxane; *C*, difference curve (A − B).

wave but only a slight shoulder at 312 mμ. If now the curve of the bicyclic analog is subtracted from that of the *D*-homoketone, a difference curve is obtained which is itself a typical "ketone wave"—of the



(CLVI)
3β-Hydroxy-5α-*D*-homoandrostan-17*a*-one



(CLVII)
trans-9-Methyl decalone-1

opposite sign to that of the decalone. Again it may be suggested that this Δ curve represents *formally* the interaction between the remote centers C-8, C-9, etc., and the 17*a*-carbonyl group in CLVI. Com-

parison of the anomalous 5α-1-oxosteroid and the *trans*-α-decalone gives a somewhat similar result, although the Δ wave is more complex.

While care is obviously necessary in the use of any such empirical approach, it seems that the extension of this "subtraction" procedure may lead to interesting correlations as more bicyclic analogs become available.

VI. Cyclohexanones: A New General Approach

A. INTRODUCTION

This section of the review represents an abridgment of a paper presently being written (Moffitt *et al.*, 168b). Some of the conclusions may be tentative, but in the author's opinion this is the first bridge between theoretical and empirical approaches to the subject.

Until recently it has been impossible to test adequately the predictions based on theoretical treatments because, solely for reasons of instrumentation, few compounds have been studied experimentally. Today, thanks to the introduction of a commercial spectropolarimeter (Rudolph) and its intensive application in the field of cyclic ketones by Djerassi and his school (1–30) dispersion curves of over 2000 ketones are available.

A recent theoretical treatment (Moffitt and Moscowitz, 168a; cf. 79, chs. 12 and 13) relates the optical activity of absorbing compounds to the interactions between the absorbing function and attached atoms and groups. In the more or less rigid cyclohexanone system the geometry of the molecule is well established, and the qualitative application of these theoretical ideas is not too difficult.

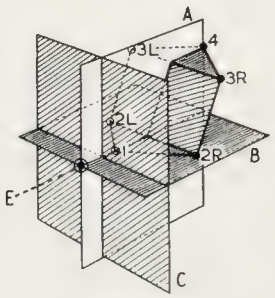
It is suggested that the best measure of the asymmetry of the surroundings of a chromophore is a function of the ellipticity called the *reduced rotational strength* (R_K). The sign of the reduced rotational strength is the same as that of the Cotton effect. Comparisons show that for compounds already studied in detail by computation (Moscowitz, 169a) the magnitude of R_K is *very roughly* proportional to the amplitude of the Cotton effect curves. In this review the amplitudes will be taken as a very approximate measure of the asymmetry of the surroundings of the carbonyl group, with full reservations regarding the theoretically unsound nature of this treatment.

This discussion is restricted to the partial rotation corresponding to the absorption band in the 300 mμ region of saturated ketones, and with the effects of alkyl substituents, which are in many cases parts

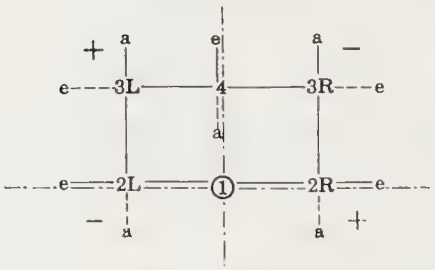
of rings other than the cyclohexanone ring itself. All data, except where otherwise stated, are from the work of Djerassi and his school, and are for solutions in methanol (D indicates a value in dioxane). The sign and magnitude of the amplitude of the Cotton effect are shown by figures in parentheses; thus (+25). These values are for $10^{-2}a$, a being expressed in terms of molecular, not specific, rotations.

B. GEOMETRY OF CYCLOHEXANONES

Cyclohexanones may be represented by the perspective formula CLVIII. This is divided into eight octants by three planes: (*a*) a vertical plane passing through O, C-1, and C-4; (*b*) a horizontal plane passing through C-1, C-2R, and C-2L (the equatorial substituents attached to C-2R and C-2L are almost but not quite in this plane); and (*c*) a vertical plane passing through the midpoint of the carbonyl bond and perpendicular to (*a*). The ring and its substituents, when projected onto a vertical plane to the right of C-4 in CLVIII are represented in CLIX, which may be called the *Octant Diagram*. Ring-



(CLVIII)
Cyclohexanone



(CLIX)
Octant projection

atoms are designated as follows: 1 = the carbonyl carbon; R_2, L_2 = the carbons α to the carbonyl (right and left, respectively); R_3, L_3 =

the β-carbons (similarly); and 4 = the γ-carbon. A bond joining a substituent to a ring carbon atom is designated by the number of its carbon atom, followed by the letter *a*, axial, or *e*, equatorial; thus, for example: R2*a*, the axial bond on right-hand carbon no. 2.

C. THE OCTANT RULE

In simple cyclohexanone derivatives all the substituents attached to the ring lie within the four octants far away from the observer's eye in CLVIII, i.e., beyond the vertical plane C.

The octant rule for cyclohexanone rotations may be stated for these simple cases as follows:

1. Atoms lying in the *far lower right* and *far upper left* octants make *positive* contributions to the partial rotations.

2. Atoms lying in the *far lower left* and *far upper right* octants make a *negative* contribution to the partial rotation.

3. Atoms lying in any of the planes dividing the octants make *no* contribution to the partial rotation.

Group 1 includes R2*a*, L3*a*, L3*e;* Group 2 includes L2*a*, R3*a*, R3*e;* Group 3 includes R2*e*, L2*e*,* 4*a*, 4*e*.

In more complex cases, some part of the molecule lies in one or more of the *near* octants, i.e., to the left of plane C in CLVIII. Atoms in each of these *near* octants have rotational contributions opposite in sign to those which they would have if they were in the corresponding *far* octants. Thus: near lower right, near upper left ...negative; near lower left, near upper right...positive.

The axial α-haloketone rule discussed previously (10,14) is now seen to be a special application of the general principles proposed here.

It must be emphasized that the treatment is based on an idealized geometry of the cyclohexanone ring (71a), and necessarily neglects many finer points of detail which are at present obscure. More detailed geometrical consideration of the structure may permit the allotment of semiquantitative asymmetry factors to the substituent atoms.

Conventional Representation of Significant Atoms

The atoms which are present in the eight octants and which make a significant contribution to the rotations may be crudely but con-

* As a first approximation.

veniently represented on a cross-shaped diagram CLX. This reproduces in a rough fashion the projection CLIX.

Upper left	Upper right
Lower left	Lower right

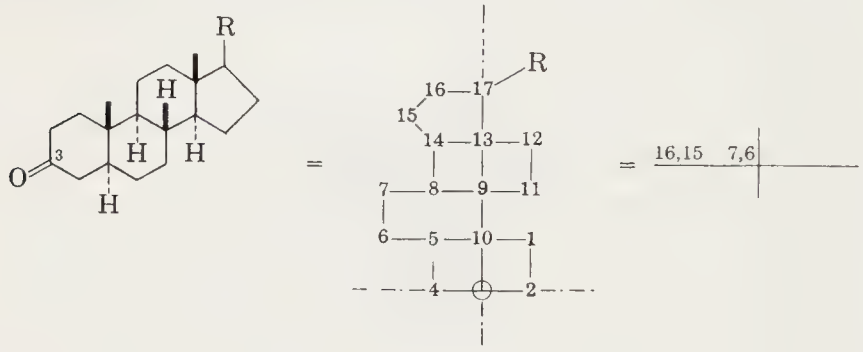
(CLX)
Octant diagram

1. Each significant atom is indicated in the appropriate segment of the diagram by its number (if a carbon atom) or by number and symbol (if not carbon) (hydrogen is neglected).

2. Atoms in the four far octants (the majority in most molecules) are indicated by *ordinary arabic* numerals; atoms in the four near octants are indicated by *italic* numerals.

3. Atoms which lie in any of the dividing planes of the octant diagram, or which cancel out (in pairs) by reason of symmetry about a dividing plane, are excluded.

For an example, see CLXI.



(CLXI)
Octant diagram for 3-oxo-5α-steroid

D. DECALONES AND STEROID KETONES

Tables XVI–XVIII present comparisons for a complete range of 9-methyldecalones and steroid ketones (both *A:B-cis* and *A:B-trans*), between the sign of the Cotton effect *predicted* by the octant rule, and the sign and magnitude *found* experimentally. It will be seen that there are no major discrepancies between predicted and found effects.

For the *cis*-decalones, the experimental data permit us to deduce the preferred conformation; these results may be compared with those predicted on conformational grounds (Klyne, 129).

TABLE XVI
Methyldecalones[a]

Position of keto group	Significant substituents[b]	Cotton effect: Predicted	Cotton effect: Found[c]
	9-Methyl-trans-*decalones* (*CLXII*)		
1	|5,6 / |9Me	+	+32 D
2	(8Me)6,7,8,9Me |	++	+52[d]
3	7,6,5 |	+	+47
4	|9Me,8,7	−−	−32[e]
	9-Methyl-cis-*decalones*		
Conformation[f]			
1 S	|	−	+51
1 NS;P	8| / |5,6 / |9Me	+	+51
2 S	|8,7,9Me	−−	unknown
2 NS	8,9Me |	++	unknown
3 S;P	|5	−	−11
3 NS	6,5 |	++	−11
4 S	9Me,7,8 |	++	+11
4 NS;P	|9Me / |5	+	+11

[a] All values are for the compounds with the angle methyl group β and the substituent in the left-hand ring (as in CLXII–CLXIV).

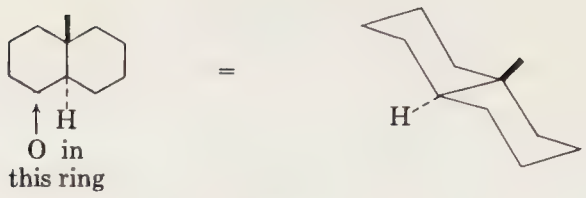
[b] Atoms not listed *either* lie in dividing planes *or* cancel one another in pairs.

[c] Amplitude: $10^{-2}a$.

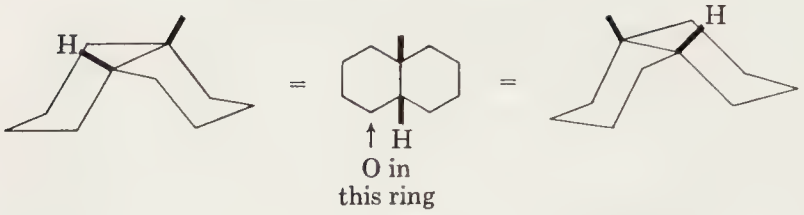
[d] With extra 8-Me group.

[e] Estimated from equilibrium mixture of *cis* and *trans*.

[f] S = steroid-like conformation (CLXIII); NS = steroid-unlike conformation (CLXIV); P = preferred conformation.

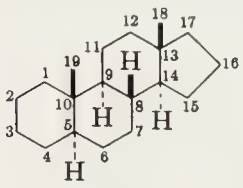


(CLXII)
trans-9-Methyldecalin

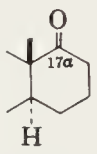


(CLXIII)
cis-9-Methyldecalin
(steroid-unlike conformation)

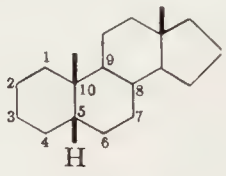
(CLXIV)
cis-9-Methydecalin
(steroid-like conformation)



(CLXV)
5-α-Steroid



(CLXVI)
D-Homosteroid



(CLXVII)
5-β-Steroid

Attention may be called to certain interesting examples. The 2-oxo-5α-steroid is a compound in which *all* significant substituents are in one octant; as expected, it has a *large* (positive) Cotton effect. The 3-oxosteroids are distinguished by a considerable measure of symmetry about the plane containing the carbonyl group, C-10, and C-19. In the 5β-isomer the number of significant substituents is reduced to a minimum by reason of the fact that the folding of the molecule brings a number of remote atoms (7, 8, 9, 11, and 18) into the horizontal carbonyl plane.

The 17α-oxo-*D*-homo-5α and 1-oxo-5α compounds are peculiar in giving R.D. curves which show little or no Cotton effect (5) and provide good illustrations of the massive effect of remote rings cancelling the effects of substituents nearer the carbonyl group. The *trans*-9-methyldecalone (CLXVIII) has a negative Cotton effect (−32), but the 17α-oxo-*D*-homosteroid (CLXIX) has a curve which shows little apparent Cotton effect. This may be rationalized when it is

TABLE XVII

Steroid Ketones (5α:A-B-*trans*) (CLXV)

Position of keto group	Significant substituents[a]		Cotton effect	
			Predicted	Found[b]
1		6,7	?	None apparent
		19,18; *12,17*[c] and *side chain*		
2	D + C rings, 7,19		+++	+121
3	16,15,7,6		+	+65
4		19, C + D rings	−−	−94
6	19	12,18,D ring	−−	−76
7		2,3	−	−16
	16,15			
11	16,18	6	+	+16
		2		
12	2,3,4,5,6		++	+42!
		18 (*side chain*)		
		D-Homoketones (CLXVI)		
17a	A + B rings		?	None apparent
	18			
17		18,12,11,19,4,3,2	−−	−96

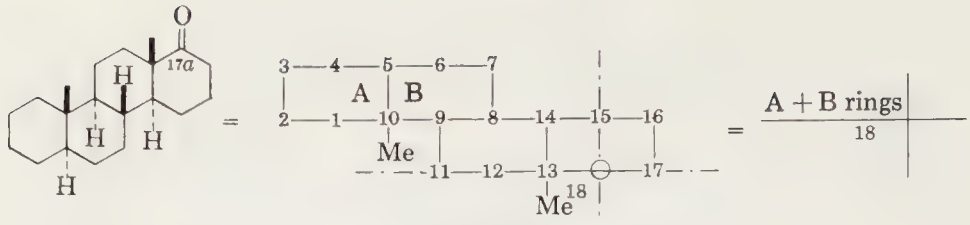
[a] Italic figures indicate atoms in near octants (cf. p. 336).

[b] Amplitude: $10^{-2}a$. The exclamation point (!) indicates that the lower wavelength extremum of the R.D. curve was not reached; i.e., that a is probably greater than the value quoted.

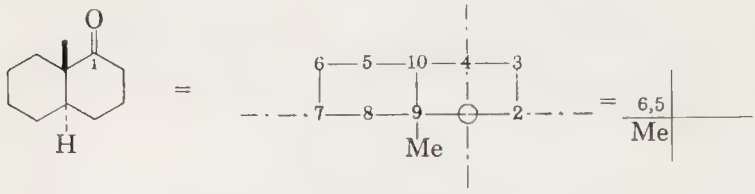
[c] 11, 13, and 16 are almost, if not exactly, in the vertical plane.

seen that practically *all of rings A and B* (C-1 to C-10) lie in the upper left (positive) quadrant.

The Octant Rule has been tested against a large number of ketones studied by Djerassi and his colleagues. With few exceptions the predictions of the rule for compounds whose molecules are simple



(CLXVIII)
9-Methyl-*trans*-1-decalone



(CLXIX)
17-α-Oxo-*D*-homosteroid

TABLE XVIII
Steroid Ketones (5β:A-B-*cis*) (CLXVII)

Position of keto group	Significant substituents[a]		Cotton effect	
			Predicted	Found[b]
1	6		−−	−136
	C + D rings			
2		C + D rings, 19	−−	Unknown
3		6	−	−27
		15,16		
4	*15,17*,18,13,14, 8,12,11,19,9		++	+3
6	19	12,18,D ring	−−	−77
	3,4			
7	11	4,3	+	+29
	16,15			
11	16,18	6	+	+15
		4,3		
12	2,3,4,5,6		+	+10
		18 (side chain)		

[a] Italic figures indicate atoms in near octants (cf. p. 336).
[b] Amplitude: $10^{-2}a$.

arrays of cyclohexane rings agree with the experimental facts as regards sign and generally as regards magnitude of the Cotton effect.

The position with bridged-ring ketones and with cyclopentanones is not yet clear; probably a semiquantitative treatment will be necessary.

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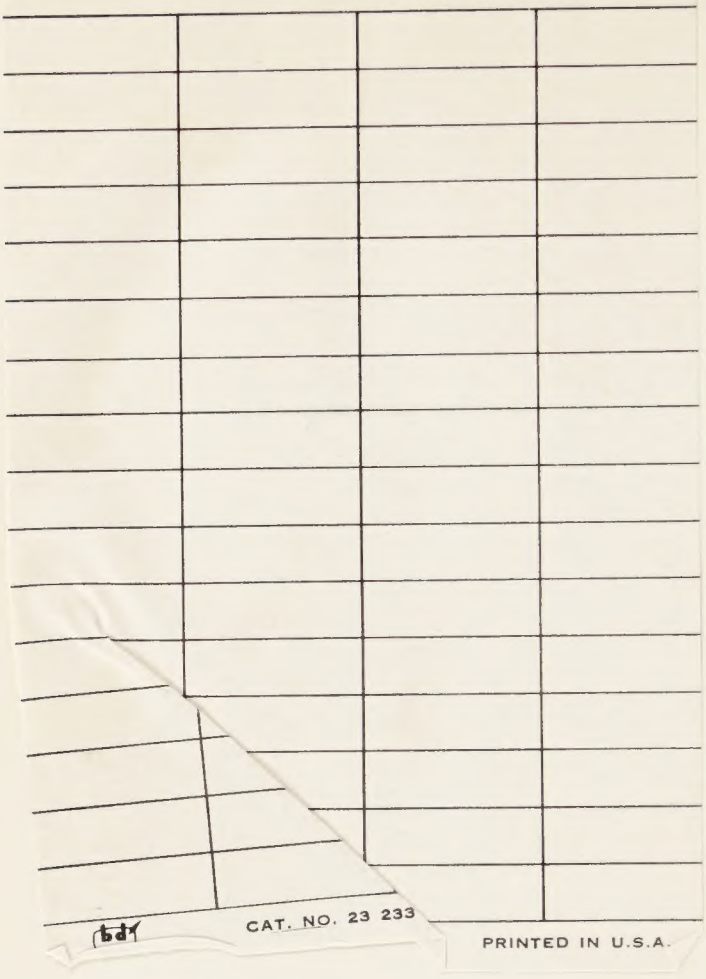

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